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EDITOR
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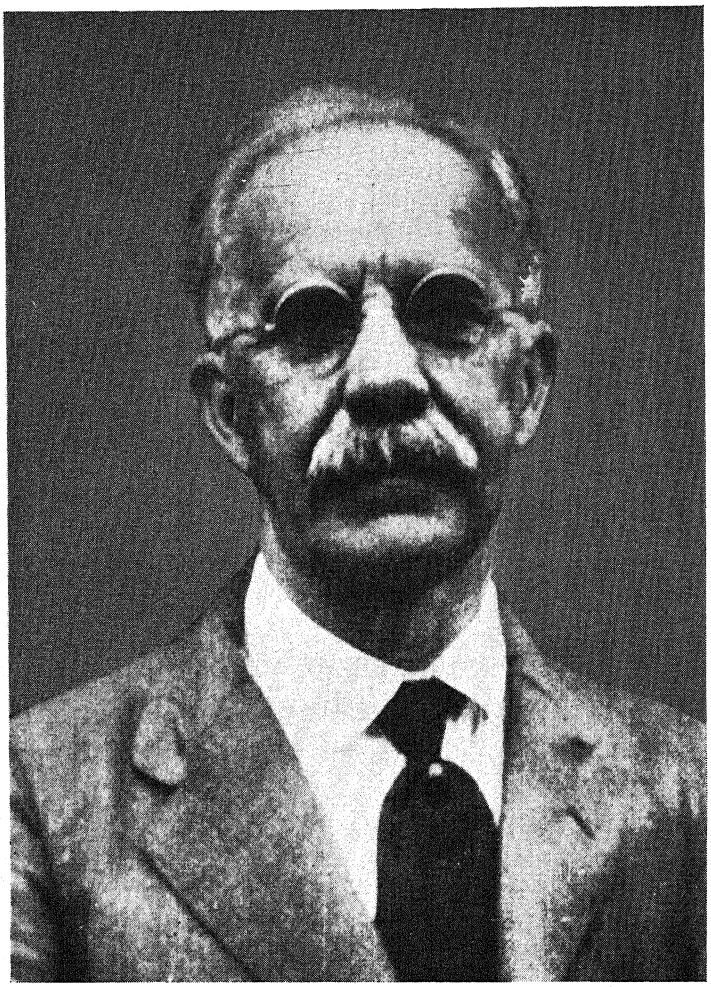
TABLE OF CONTENTS

		PAGE
William Jesse Goad Land (with portrait)	*C. J. Chamberlain*	1– 2
Gross morphology and histology of developing fruit of the apple	*H. B. Tukey and J. Oran Young*	3– 25
Effects of growth-regulating substances on shoot development of roses during common storage	*Paul C. Marth*	26– 49
Susceptibility of *Colchicum* and *Chlamydomonas* to colchicine	*Ivor Cornman*	50–62
Biochemical nitrogen fixation studies. IV	*F. E. Allison, S. R. Hoover, and F. W. Minor*	63– 71
Microsporogenesis and development of seed in *Lobelia cardinalis*	*George O. Cooper*	72– 81
Volatile sulphur content of black mustard plants	*Scott V. Eaton*	82– 89
Cell size in developing ovaries of *Iris fulva*	*H. P. Riley and Dorothy Morrow*	90– 98
Effects of photoperiod on microsporogenesis in Biloxi soybean	*C. S. Nielsen*	99–106
Cytology of apogamy in *Polysticum tsussemense*	*Sister Mary T. Patterson*	107–114
Effects of growth substances on reserve starch	*S. C. Bausor*	115–121
Chloroplast substance of spinach leaves	*C. L. Comar*	122–127
Physiological studies on mosses. IV	*S. L. Meyer*	128–132
Effects of light quality on growth and mineral nutrition of bean	*L. M. Rohrbaugh*	133–151
Device for measuring entry of water into roots	*H. E. Hayward, Winifred M. Blair, and P. E. Skaling*	152–160
Effects of platinum chloride on bean and tomato	*Charles L. Hamner*	161–166
Development of the node in *Ricinus communis*	*Mary E. Reynolds*	167–170
Variations in chromosomal association and behavior during meiosis among plants from open-pollinated populations of *Dactylis glomerata*	*W. M. Myers and Helen D. Hill*	171–177
Influence of controlled-atmosphere storage on respiration of McIntosh apples	*Robert M. Smock*	178–184
Development of the macrogametophyte of *Miersia chilensis*	*Marion S. Cave*	185–187
Potassium and phosphate nutrition of pineapple in relation to nitrate and carbohydrate reserves	*G. T. Nightingale*	191–223
Growth substances and dormancy of spores of *Phycomyces*	*W. J. Robbins, Virgene W. Kavanagh, and F. Kavanagh*	224–242
Histological responses of *Vicia faba* to indoleacetic acid	*Barbara F. Palser*	243–263
Studies in Californian Hepaticae. III	*A. W. Haupt*	264–272
Spindle abnormalities in *Mentha*	*C. P. Swanson and Ray Nelson*	273–280
Comparative anatomy of synthetic auxins and derivatives	*G. S. Avery, Jr., J. Berger, and B. Shalucha*	281–287
Anatomy and histology of the Eureka lemon	*E. S. Ford*	288–305
Influence of sulphur deficiency on metabolism of black mustard	*Scott V. Eaton*	306–315
Nutritional effects of boron on growth and development of the sunflower	*Benjamin Lowenhaupt*	316–322
Differentiation of avocado blossom buds in Florida	*Philip C. Reece*	323–328

		PAGE
Optimal conditions for the hydrolysis of arabogalactan by *Aspergillus niger*	*E. J. Ratajak and H. S. Owens*	329–337
Treatment of seeds with synthetic growth-regulating substances	*W. S. Stewart and C. L. Hamner*	338–347
Chemical changes accompanying growth and development of seed and fruit of the Elberta peach	*F. A. Lee and H. B. Tukey*	348–355
Histological study of freezing, desiccation, and winter injury of raspberry canes	*A. O. Simonds*	356–361
Effects of vaporous naphthoxyacetic acid on development of tomato fruits, with special reference to their vitamin C content	*J. W. Mitchell and Muriel R. Whitehead*	362–365
Charles Joseph Chamberlain (with portrait)	*J. T. Buchholz*	369–370
Vegetation and substrate of Cranberry Glades, West Virginia	*H. C. Darlington*	371–393
Survey of anatomy, ergastic substances, and nuclear size in *Echinocystis macrocarpa*	*Flora M. Scott*	394–408
Translocation of the floral stimulus in *Xanthium*	*Alice P. Withrow and R. B. Withrow*	409–416
Nature and rate of development of root system of *Convolvulus arvensis*	*J. C. Frazier*	417–425
Developmental anatomy of the fruit of the Deglet Noor date	*E. M. Long*	426–436
Development of sorus and prothallium of *Acrophorus stipellatus*	*Betty F. Thomson*	437–442
Anatomical studies of the roots of juvenile lodgepole pine	*R. J. Preston, Jr.*	443–448
The chlorophyll-protein complex. II	*L. S. Moyer and M. M. Fishman*	449–454
Effects of vitamins on germination and growth of orchids	*G. R. Noggle and F. L. Wynd*	455–459
Transplantation experiments in peas. III	*F. W. Went*	460–474
Effects of application of thiamin to *Cosmos*	*James Bonner*	475–479
Relationship of dissociation of cellular proteins by incipient drought to physiological processes	*Henry T. Northen*	480–485
Defoliation of tomato plant as a response to gaseous emanations from the fruit	*John Skok*	486–489
Varietal differences in anatomy of cross-section of wheat grain	*J. C. Bates*	490–493
Effects of micro-nutrient deficiencies on growth and vitamin content of the tomato	*C. B. Lyon, K. C. Beeson, and G. H. Ellis*	495–514
Effects of solvent upon absorption spectra of chlorophylls *A* and *B;* their ultraviolet absorption spectra in ether solution	*D. G. Harris and F. P. Zscheile*	515–527
Cyto-genetic consequences of X-ray treatment of pollen in *Petunia*	*C. M. Rick*	528–540
Analysis of variance and covariance of chromosomal association and behavior during meiosis in clones of *Dactylis glomerata*	*W. M. Myers*	541–552
Cytological studies in relation to the classification of the genus *Calochortus*. III	*J. M. Beal and Marion Ownbey*	553–562
Meiosis in autotetraploid *Secale cereale*	*J. G. O'Mara*	563–575

		PAGE
Composition of the tomato plant as influenced by nutrient supply in relation to fruiting	*D. I. Arnon and D. R. Hoagland*	576–590
Effects of nutrient-solution concentration on the growth of *Marchantia polymorpha*	*P. D. Voth*	591–601
Chromosome numbers in the subfamily Aurantioideae, with special reference to the genus *Citrus*	*C. A. Krug*	602–611
Influence of temperature on photoperiodic reactions in leaf blades of Biloxi soybean	*M. W. Parker and H. A. Borthwick*	612–619
Occurrence of aneuploids in *Lilium*	*R. N. Stewart*	620–626
Cytology of the autotetraploid rye	*T. C. Chin*	627–632
Role of pyrenoids in algae and of vacuoles in plastids of higher plants and in fungi	*S. R. Bose*	633–638
Effects of growth-regulating substances on a parthenocarpic fruit	*H. E. Clark and K. R. Kerns*	639–644
Nucleolar stain and nucleal reaction	*C. S. Semmens*	645–649
Pollen-tube development in *Taraxacum officinale*	*Elaine J. Katz*	650
CURRENT LITERATURE		188, 366, 494, 651

For titles of book reviews see index under author's name



VOLUME 104 NUMBER 1

THE
BOTANICAL GAZETTE

September 1942

WILLIAM JESSE GOAD LAND

(WITH PORTRAIT)

WILLIAM JESSE GOAD LAND was born at Alton, Illinois, December 2, 1865. He studied at Rome (Indiana) Academy, 1888–92; received his S.B., University of Chicago, 1902, and Ph.D., 1904. He was Principal of Rome Academy, 1893–94; Principal of the High School, Grand Rivers, Kentucky, 1894–95; Superintendent of City Schools, Ashley, Illinois, 1895–1901. At the University of Chicago he was Fellow in Botany, 1903; Assistant in Morphology, 1904–06; Associate, 1906–08; Instructor in Botany, 1908–11; Assistant Professor, 1911–15; Associate Professor, 1915–28; Professor, 1928, until he retired as Professor Emeritus, 1931. He was Fellow, American Association for the Advancement of Science; Member, Botanical Society of America, Illinois Academy of Science, American Microscopic Society, Deutsche Botanische Gesellschaft, Texas Geological Society, Texas Academy of Science, Phi Beta Kappa, Sigma Xi, Alpha Sigma Phi, and National Rifle Association.

Dr. LAND's lifelong connection with the University of Chicago began in 1898 with three courses in botany by correspondence, while he was Superintendent of Schools at Ashley, Illinois. He must have been an indefatigable worker, for he was teaching many subjects and making scientific apparatus, in addition to the correspondence study. For that study he made a simple but efficient camera lucida. Professor CHARLES R. BARNES once remarked that DR. LAND could do more things than any other man he ever knew.

He first came to the campus of the University for the Summer Quarter of 1900. In the second term of that quarter, since there were no botanical courses suitable for him, Dr. JOHN M. COULTER suggested that he undertake a piece of research. NAVASHIN had just announced double fertilization in the lily, and that summer Dr. LAND demonstrated it convincingly for a dicotyledon. His next paper was a morphological study of *Thuja occidentalis*. Then there was a study of the life history of *Torreya* (with JOHN M. COULTER), and—also with Dr. COULTER—a paper on *Lepidostrobus*, and one on the use of the cotyledon in phylogenetic studies. He had articles on the Hepaticae, some of them with Professor BARNES. He also published papers on laboratory apparatus and technique. Among his important contributions were those on *Ephedra*. His main work, however, was a book on the morphology and

phylogeny of the Hepaticae. For this he collected material in various parts of the United States, Mexico, and the South Seas, and undertook extensive research.

He was an able and enthusiastic teacher. He collected his own material for class work because he knew just what he wanted. He often quoted FRANKLIN'S "If you want your business done, go; if not, send." Any student who wished to know how to prepare laboratory material was taught even photography and photomicrography, besides the latest in microtechnique. He developed the formalin-acetic-alcohol and the formalin-acetic-water fixing agents, which, with various modifications, are still standard. The Spencer rotary microtome, invented by Dr. H. N. OTT, owes much to Dr. LAND'S criticism, suggestion, and encouragement.

While he believed that in a morphological course there should be both lecture and laboratory work, he was convinced that the lecture feature was overemphasized. He laid great stress upon laboratory work and always planned it to develop observation and initiative.

Aside from his academic work, Dr. LAND was extremely patriotic and an energetic believer in preparedness. He was a master shot with rifle and revolver, and was elected a life member of the National Rifle Association, winning decorations on both Army and Navy ranges. During the first World War he organized the University of Chicago Rifle Club and built an excellent rifle range at the University. Dr. LAND invented the holder for the sniper's telescope sight used by our Army and Navy, giving it to the government without charge.

Dr. and Mrs. LAND spent most of their time after his retirement at their home in Brownsville, Texas, but came each summer to their home at Rome, Indiana. Dr. LAND passed on at this summer home on August 1, 1942. Mrs. LAND has the sympathy of a host of his colleagues and students, who will long cherish his memory.—C. J. CHAMBERLAIN, *University of Chicago.*

GROSS MORPHOLOGY AND HISTOLOGY OF DEVELOPING FRUIT OF THE APPLE[1]

H. B. TUKEY AND J. ORAN YOUNG

(WITH SIXTY-NINE FIGURES)

Introduction

Individual aspects of the fruit of the apple have been studied by a number of workers, including gross morphology by KRAUS (11); gross development by WHITEHOUSE (28); variations in internal structure by KRAUS (12); vascular anatomy by KRAUS and RALSTON (13) and MACARTHUR and WETMORE (15); nature of inflorescence and fruit by BLACK (5); anatomy and phylogenetic concept by MACDANIELS (17); cytology and anatomical development by TETLEY (21, 22); nature of lenticels by CLEMENTS (6); protective layers of fruit by BELL (3, 4); changes in chemical composition of developing apples by ASKEW (2); structure of flesh in relation to growth and senescence by SMITH (19); fertilization and early development of embryo by ELSSMANN and VON VEH (7), STEINEGGER (20), and SEN (18); and relation of seed to fruit development by EWERT (8, 9). More recently MACARTHUR and WETMORE (16) have analyzed development of fruits of the McIntosh Red and Wagener apples. This paper, while overlapping some of these studies in certain details, attempts to give a more or less connected picture of the gross development and the principal tissue changes in the fruit of the apple in the western New York fruit area of the United States, from 1 month before full bloom throughout the growing season to fruit ripening.

Material and methods

Five varieties of apple, ripening at intervals from very early season to late season—Lodi (very early), Early Harvest (early), Twenty Ounce and McIntosh (mid-season), and Rome (late)—were used all or in part during five growing seasons as follows:

1. Season of 1930, variety Lodi, fixed in chromacetic April 10, 1 month before full bloom, material obtained through the courtesy of Dr. B. R. Nebel.

2. Season of 1934, varieties Early Harvest, McIntosh, and Rome, fixed in formalin-acetic-alcohol on Mondays, Wednesdays, and Fridays from May 23 to fruit ripening (August 3, Spetember 17, and October 31, respectively).

3. Season of 1935, variety Twenty Ounce, fixed in Karpechenko's chromacetic at 2- and 3-day intervals from May 14 to June 10.

4. Season of 1938, variety Twenty Ounce, cleared in phenol-lactic-glycerin on Mondays, Wednesdays, and Fridays from May 17 to September 28.

5. Season of 1940, varieties McIntosh and Twenty Ounce, fresh material.

All samples were collected in the orchards of the New York State Agricultural Experiment Station at Geneva, New York. The wide range of varieties,

[1] Journal Paper no. 469 of the New York State Agricultural Experiment Station.

the frequency of sampling, and the several seasons included have made it possible to observe the variations in the details studied, as well as the general trends.

Samples cleared in phenol-lactic-glycerin for gross structural measurements were cut into thin slices with a razor or sharp knife, then placed and held in the following solution: phenol 20 gm., lactic acid 20 gm., glycerin 40 gm., water 20 gm. Although this mixture does not clear the sections as much as cedar oil, the vascular structures, core line, and cartilaginous tissues can be seen plainly. Definition is sufficiently sharp for satisfactory photographing (figs. 1, 2). The phenol-lactic-glycerin method has the advantages of rapidity and inexpensiveness, making observations on masses of material possible. Further, the tissues do not become brittle as in xylol or cedar oil but remain flaccid. For example, it was possible to separate the epidermis and hypodermal layer from the underlying tissues, to mount them, and to make drawings of the cells in surface view.

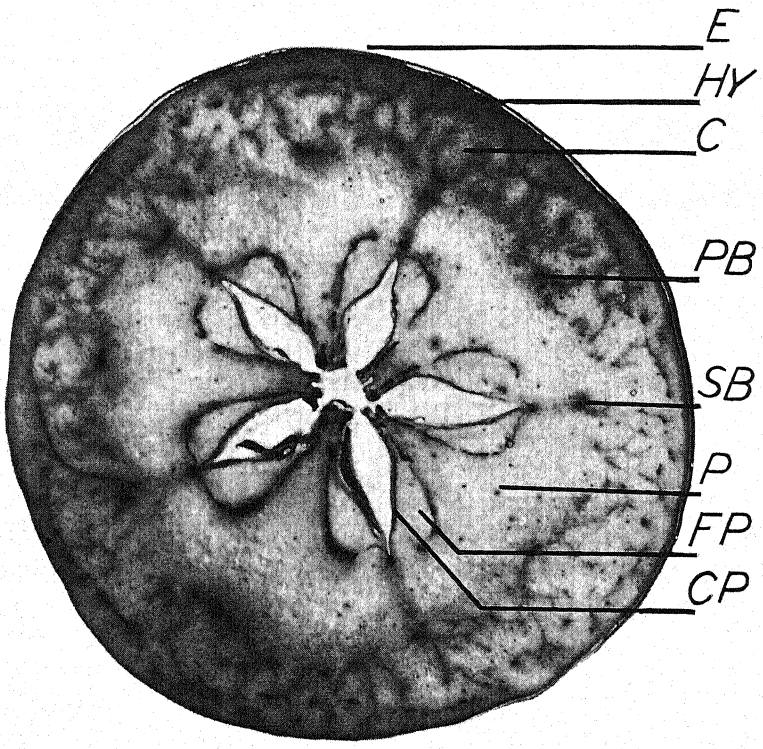


FIG. 1.—Cross-section of Twenty Ounce apple cleared in phenol-lactic acid, showing parts discussed. *e*, epidermis; *hy*, hypodermal layer; *c*, cortex; *pb*, petal bundle; *sb*, sepal bundle; *p*, pith; *fp*, fleshy pericarp; *cp*, cartilaginous pericarp.

For detailed studies of early stages when the material was still small, samples were fixed in Karpechenko's chromacetic and run up into paraffin for sectioning on a rotary microtome. The sections were

stained with safranin, crystal violet, and orange G. For detailed studies of later stages, when the fruits were larger, samples were fixed in a solution containing 23 cc. of formalin, 67 cc. of glacial acetic acid, and 100 cc. of 50 per cent alcohol, and sections were made directly either freehand or on a sliding microtome. Despite the progressive hardening of cartilaginous portions of the carpels, it was possible to make reasonably complete sections of fruits up to $2\frac{1}{2}$ inches in diameter. These sections were in some cases 100 μ in thickness yet were thin enough to show clearly the essential histological features after staining in safranin and light green.

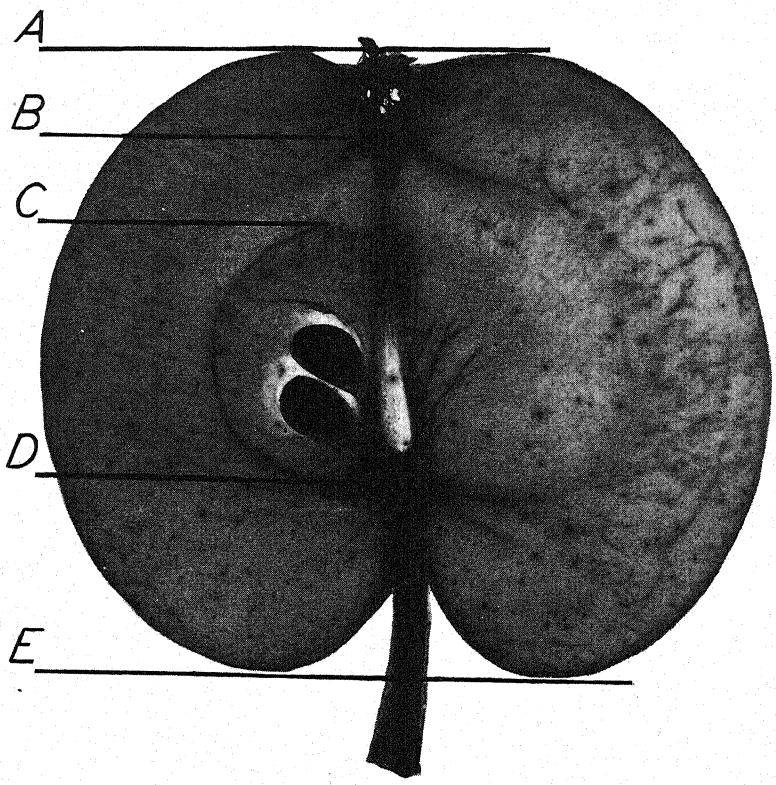


FIG. 2.—Longisection of Twenty Ounce apple cleared in phenol-lactic acid. *A–E*, length of fruit; *B–D*, length of carpel; *C–D*, length of carpel "blade."

MEASUREMENTS

Gross measurements of parts indicated in figures 1 and 2 were made from fresh material collected every Monday, Wednesday, and Friday from pre-bloom to fruit ripening as follows:

Weight.—Average from ten specimens for early stages and two to five for later in season.

Volume.—Average water displacement from ten specimens for early stages and two to five for later.

Transverse diameter.—Average greatest cross-sectional diameter.

Polar diameter.—Average over-all length of fruit.

Sepal and petal bundle diameters.—Average diameter of circles drawn through the five sepal bundles and five petal bundles in cross-section of fruit.

Core line diameter.—Average inside diameter of core line as measured by averaging ten points on core line in cross-section of fruit.

Core diameter.—Average diameter of circle drawn through the five dorsal carpellary bundles in cross-section of fruit.

Carpel length.—Average length from base of style to region of anastomosis at proximal end of pedicel (fig. 2*B–D*).

Carpel blade length.—Average length of "blade" portion of carpel in longitudinal section (fig. 2*C–D*), considering carpel as a leaf.

Carpel suture diameter.—Average greatest diameter from dorsal to ventral suture in cross-section of fruit.

Carpel cheek diameter.—Average greatest diameter of cartilaginous portion of carpel at right angles to suture diameter in cross-section of fruit.

Flesh carpel wall diameter.—Average greatest diameter of fleshy portion of carpel in cross-section of fruit.

Nucellus and integument length.—Average over-all length of nucellus and integuments.

Embryo length.—Over-all length of embryo.

Of these measurements, some were found to have greater significance and to be less variable than others. For example, a circle drawn through the petal bundles is slightly smaller than one drawn through the sepal bundles, and this in turn is smaller than a circle through ten points on the core line; but the relationship appears to be of no essential significance so far as this study is concerned. The total length of the carpel is so altered by the size of the tip or of the base as to mask the true increase in size of its main portion. For this measurement the carpel blade served best. The carpel cheek diameter varied with the number of seeds and with the degree of spreading of the carpel, so that it was not useful. For a measure of the development of the fleshy portion of the carpel it was found best to use the greatest thickness of the wall from the cartilaginous pericarp to the periphery of the fleshy carpel. On the other hand, diameters of entire fruit, core line and core, and fleshy portion of the carpel wall proved good measurements in transverse section, as did over-all length of fruit, carpel blade length, and over-all length of nucellus and integuments and embryo in longitudinal sections.

Permanent outline drawings of the samples fixed in formalin-acetic-alcohol were made in both cross and longitudinal section by outlining the principal parts and tissues with an indelible pencil and transferring to filing cards by stamping the cross-sections upon them. These were used to compare shrinkage with the formalin-acetic-alcohol measurements whenever individual fruits of this material were studied. Although compensation was made for shrinkage on this basis, little occurred.

Curves were drawn by use of a Keuffel and Esser spline and spline weights, which were found highly satisfactory in the drawing of smooth curves with a minimum of departure from the points on the graph.

Outline drawings were made by tracing the images of the cleared sections as projected on a ground-glass camera plate. This method facilitated rapid and accurate tracing of the larger structures at constant magnification. Early Harvest and Twenty Ounce were chosen for this portion of the study, since KRAUS (12) has shown that the fleshy portion of the carpel is relatively large in these varieties. All drawings of histological details were made at constant magnification with the aid of a camera lucida.

Measurements of cell size were made with the aid of a sliding ocular micrometer in combination with a 4-mm. objective.

General consideration of tissues

The morphological interpretation of the apple fruit has long constituted a theoretical controversy. There are two divergent lines of thought: (*a*) that the apple consists of five drupelike carpels contained within the fleshy torus or receptacle and is therefore in large part a fleshy development of the stem or axis; (*b*) that the fleshy portion of the apple, exclusive of the inclosed carpels, is composed of the fused and enlarged bases of the floral appendages and represents the floral tube. The first theory is based largely on ontogenetic observation, while the second is supported in the main by phylogenetic considerations.

The receptacular theory, as expounded by KRAUS (11, 13) and later by BLACK (5), holds that the five carpels are composed of a cartilaginous endocarp and a relatively thin but highly variable fleshy exocarp. The "core line" of the apple is interpreted as representing a cambial region between the cortex and pith. The appendicular theory as stated by MACDANIELS (17) interprets the core line as "representing the line of fusion between the floral tube and the ovary." While it is not the purpose of this paper to enter the discussion on one side or the other, yet the interpretation of KRAUS has seemed to the writers more correctly to describe the structures observed, and the terminology used here is in accordance with the receptacular theory.

Figures 1 and 2 illustrate a Twenty Ounce apple in transverse and longitudinal section, with the parts labeled which are mentioned in this paper.

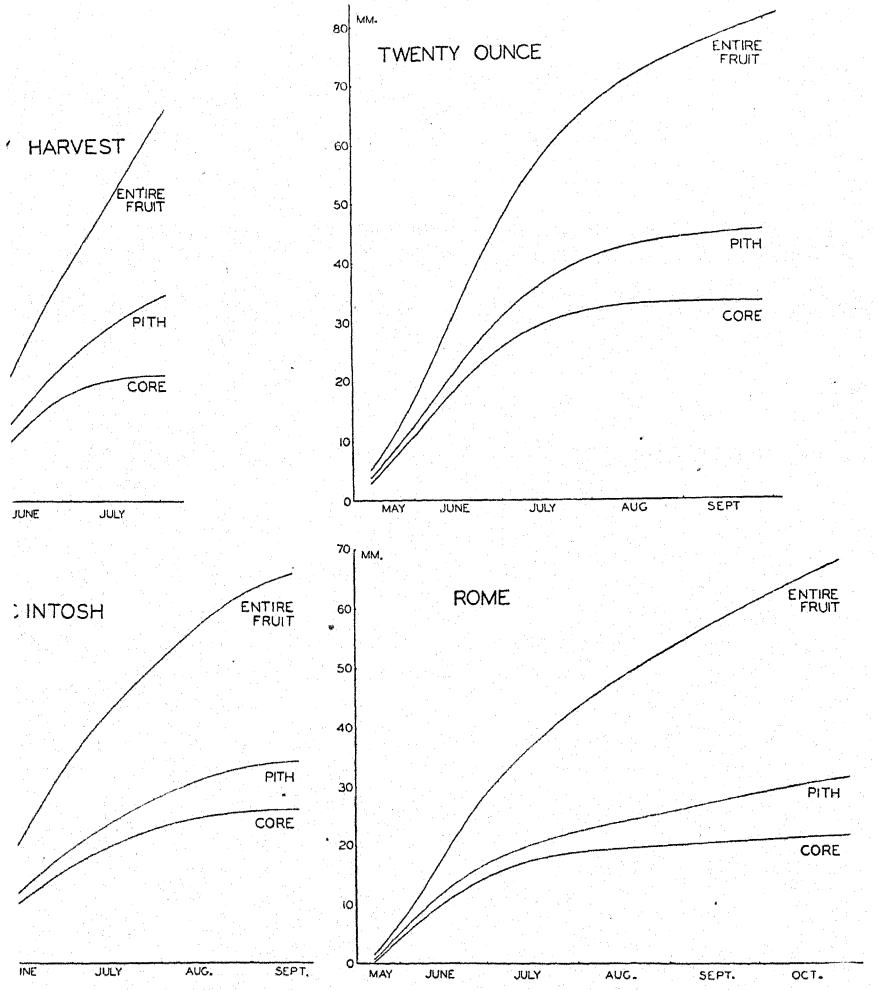
Results

GROSS DEVELOPMENT

The increases in transverse diameter of the fruit, pith, and core or outer boundary of the pericarp are charted in figure 3 for Early Harvest, Twenty Ounce, McIntosh, and Rome varieties and show the relation between these parts during development. The distance between the curve for the entire fruit and that for the pith gives a measure of the increase of cortical tissue; the distance between the curve for the pith and that for the pericarp gives a measure of the increase of the pith region; while the interval between the curve for the pericarp and the base line gives a measure of the increase of the core region.

The first portions of the curves are similar for all varieties but differ farther along. For the variety ripening in early summer (Early Harvest), the curve for the entire fruit is almost a straight line, but for each successively later-ripening variety the curve flattens as the season progresses (28). This may be associated with sunlight (22), or temperature or moisture (2); but that it is—in part at least—a varietal characteristic of slower growth rate is shown by superimposing the curves up to August 10. The earliest ripening variety shows a more rapid rate

ut the entire growth period, for each successively later ripenty the rate falls off at an earlier less pith, thus compensating for the much less increase in the pericarp. MacArthur and Wetmore (15) report similarly.



Increase in transverse diameters of entire fruit, pith, and "core" or outer boundary of pericarp rvest, McIntosh, Twenty Ounce, and Rome apples from full bloom to fruit ripening.

of the pith follows somewhat trend as that for the cortical it the curve is less steep, where- of the pericarp is completed early. In other words, the ne of growth for the entire fruit o in later season of an increasrtion of cortex and somewhat

Volume increase (fig. 4) presents a truer picture of total growth than does diameter increase. The curves of the three varieties reflect the same general trend as the curves for diameter, in which the early season growth of the early-ripening Early Harvest is more rapid than that of McIntosh, and the

latter in turn is more rapid than that of the late-ripening Rome.

While the curves approach a straight line, as reported commonly in horticultural literature for increase in volume of apple fruits in various parts of the country, there is nevertheless a slight tapering off of growth in McIntosh and Rome as the time of harvest is reached. If an explanation is needed it may lie in the fact that New York State is not an irrigated section, and that fall temperatures at harvest are relatively lower than in the west and south, from which most of the straight-line curves have been reported.

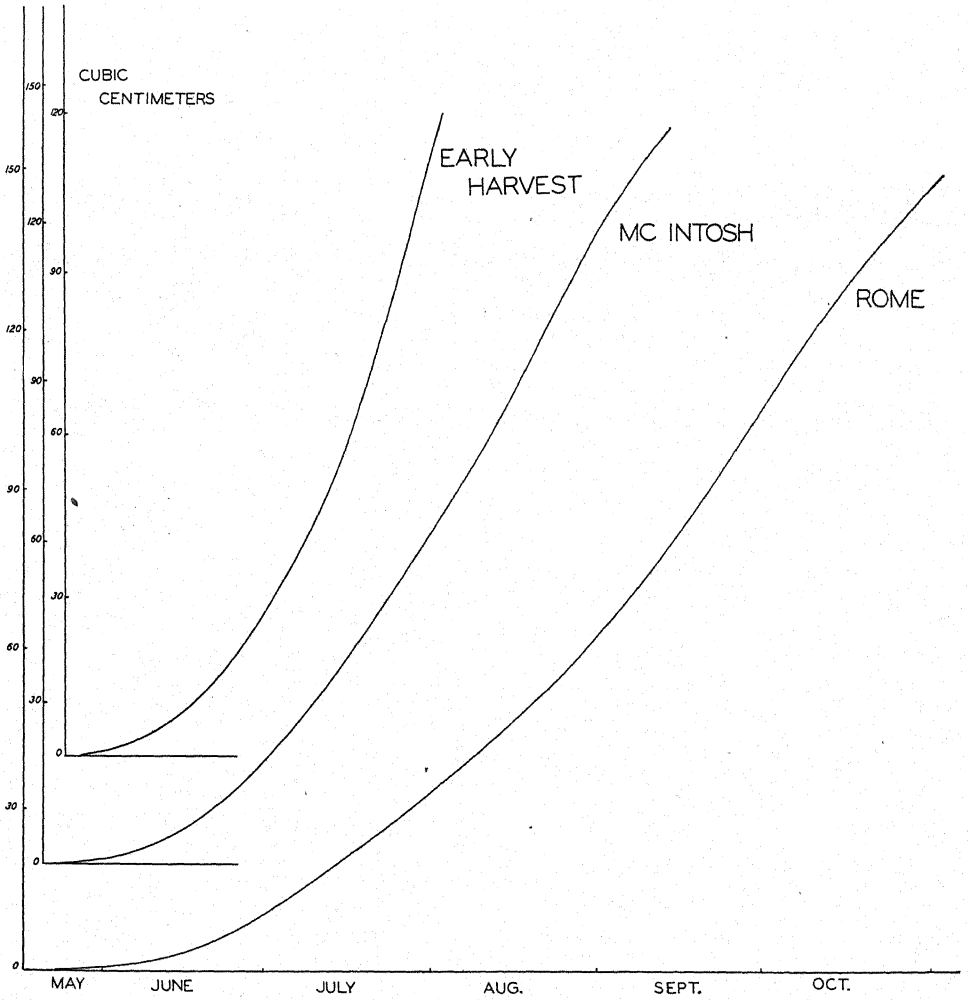
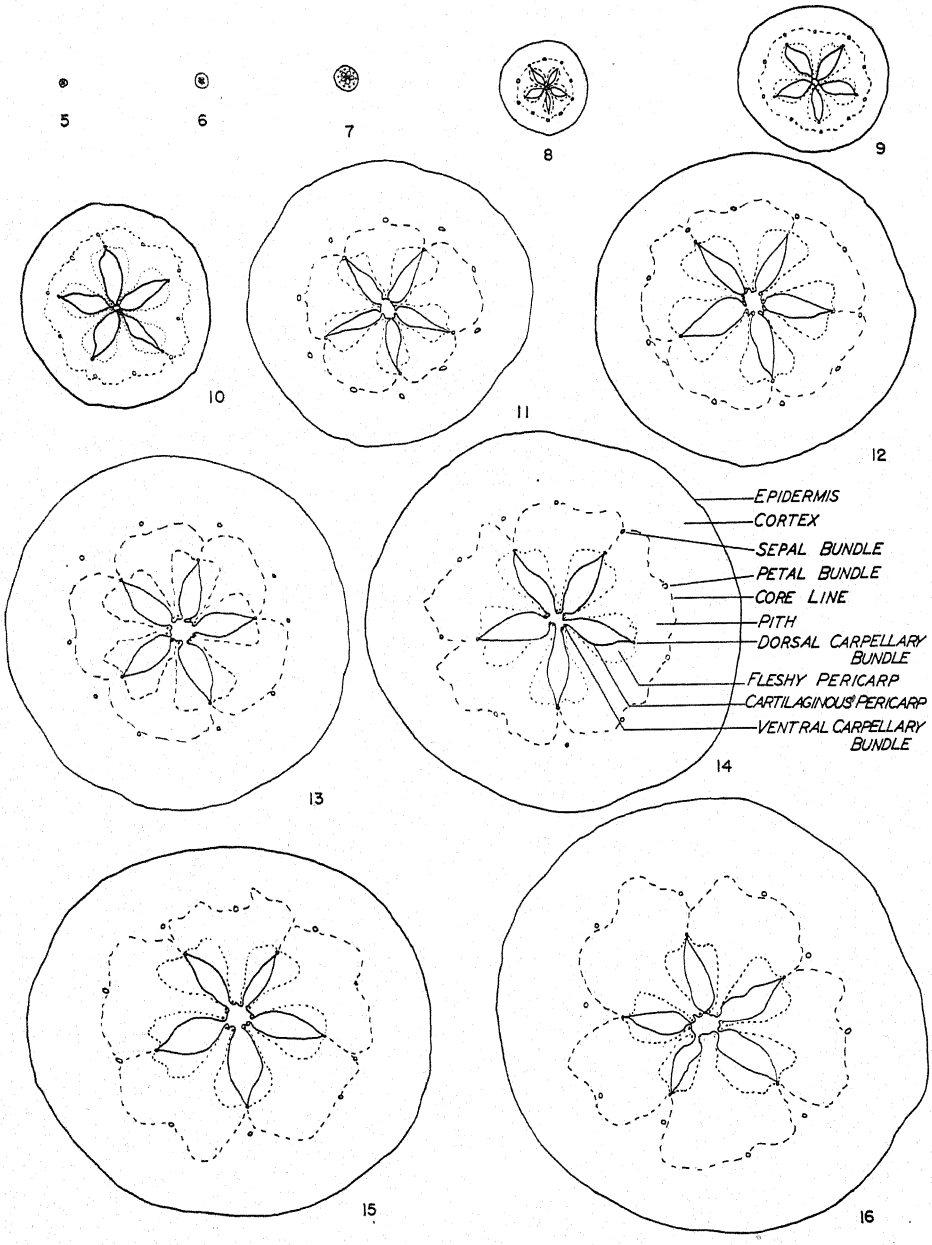
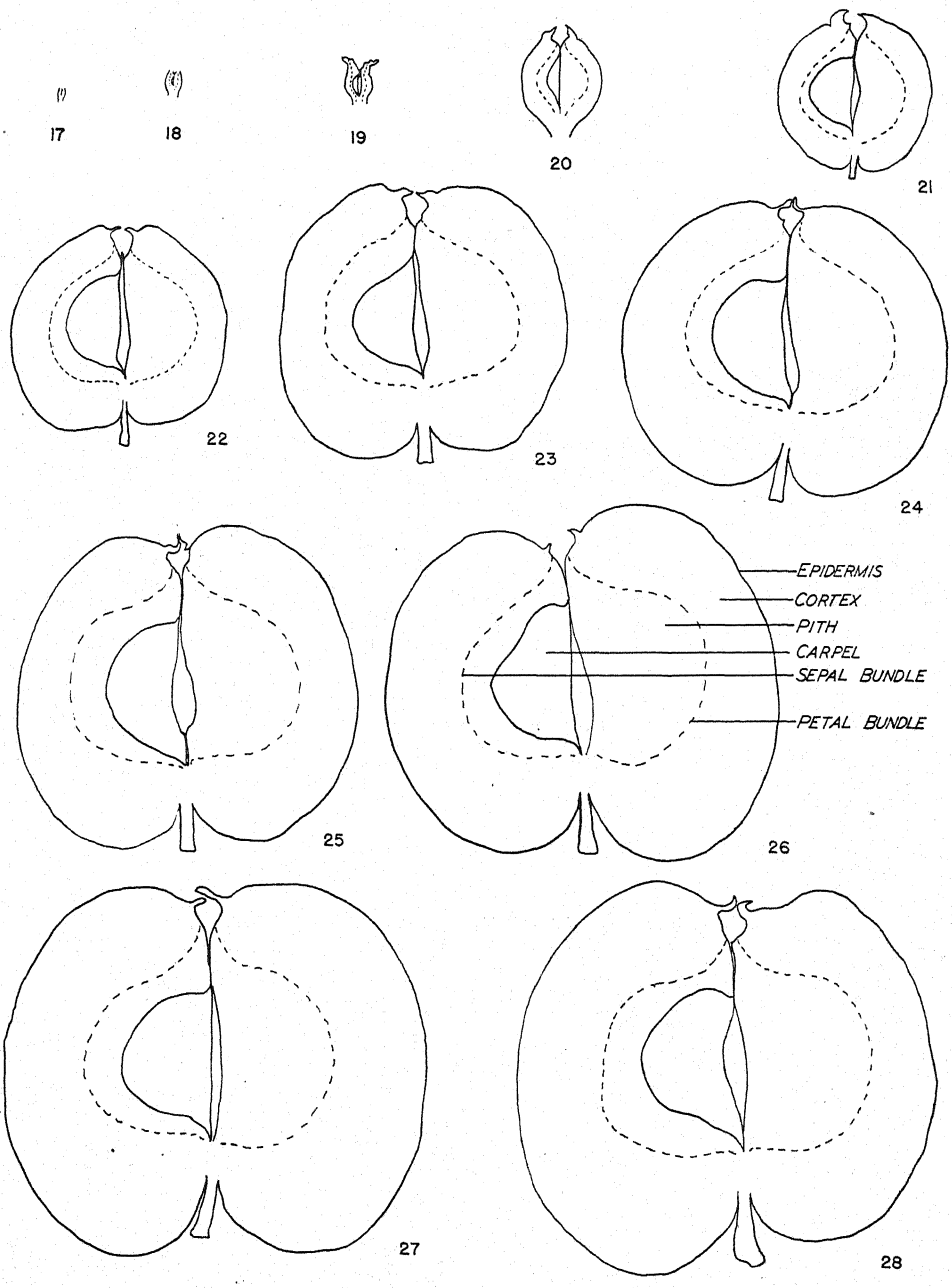


FIG. 4.—Increase in volume of fruit of Early Harvest, McIntosh, and Rome apples from full bloom to fruit ripening (water displacement method).

Figures 5–28, of the Twenty Ounce apple at 2-week intervals from 1 month before full bloom to fruit ripening, show the relation between the different parts of the developing fruit and also several features not seen in the curves of gross



FIGS. 5–16.—Tracings of Twenty Ounce apple in cross-section from 4 weeks before full bloom to fruit ripening: Fig. 5, 4 weeks before full bloom; fig. 6, 2 weeks before full bloom; fig. 7, full bloom; fig. 8, 2 weeks after full bloom; fig. 9, 4 weeks; fig. 10, 6 weeks; fig. 11, 8 weeks; fig. 12, 10 weeks; fig. 13, 12 weeks; fig. 14, 14 weeks; fig. 15, 16 weeks; fig. 16, 18 weeks (ripe).



FIGS. 17–28.—Tracings of Twenty Ounce apple in longisection from 4 weeks before full bloom to fruit ripening: Fig. 17, 4 weeks before full bloom; fig. 18, 2 weeks before full bloom; fig. 19, full bloom; fig. 20, 2 weeks after full bloom; fig. 21, 4 weeks; fig. 22, 6 weeks; fig. 23, 8 weeks; fig. 24, 10 weeks; fig. 25, 12 weeks; fig. 26, 14 weeks; fig. 27, 16 weeks; fig. 28, 18 weeks (ripe).

development. The cartilaginous portion of the carpels develops rapidly in June or early July, reaching maximum size in transverse direction (figs. 5–16) earliest of any of the tissues making up the bulk of the fruit. The fleshy portion of the carpels continues growth in late July and early August and is the next to reach maximum size. The pith and cortical region, on the other hand, continue growth through August and September and make up the bulk of the apple at fruit ripening.

The edges of the carpels are in approximate contact along the ventral suture until about 4 weeks after full bloom. This condition is described by horticulturists as a closed core. In Twenty Ounce the edges of the carpel spread apart along the ventral suture and curve away from the central axis of the fruit. The edges of the carpels are no longer in contact throughout their length, resulting in the characteristic "open core" of the variety as noted by MACARTHUR and WETMORE (15) and others (figs. 5–16). The core accordingly becomes larger, but the size of the individual carpels remains the same, except for the development of parenchymatous tissue in the region of the ventral and carpellary bundles and for the increase in size of the fleshy portion of the carpel.

In longitudinal section (figs. 17–28) the carpel also reaches maximum length by mid-summer (22). The pith, however, increases relatively less in length than in transverse diameter. In the cortical region there is a comparably greater increase in length, owing to a greater upward and radial direction of growth of the cortical cells at the apex and a corresponding downward and radial growth of these cells at the base (15), so that the stem may be submerged in the cavity which surrounds it.

HISTOLOGICAL DEVELOPMENT

Except when otherwise noted, the histological observations were made on the McIntosh variety. Measurements of size and numbers of cells in the different tissues are given in tables 1 and 2. Histological details at monthly intervals from 1 month before full bloom to fruit ripening are shown in figures 34–63.

CARPEL.—Development of the carpel resembles somewhat that of a drupe fruit (10, 14, 27). At fruit ripening it is composed of inner epidermis, cartilaginous pericarp, fleshy pericarp, and outer epidermis.

Over the full surface of the carpels extends a continuous layer of epidermis. The greater extent of this layer represents the inner epidermis, but in the cleft between adjoining carpels the surface cells may properly be called outer epidermis (figs. 31, 33). The outer epidermis cannot be identified in the region between the fleshy pericarp and the pith. In Twenty Ounce the epidermal cells lining the cleft between two carpels may become much enlarged and pressed together, so that the cleft is distinguishable only by microscopic observation (figs. 29–33).

Four weeks prior to full bloom the cells of the inner epidermis are somewhat radially elongate, about $10 \times 5\ \mu$ in transverse section (figs. 34–39). At full bloom they are further radially elongate and form a palisade-like layer of cells averaging $20\ \mu$ in radial direction by $8\ \mu$ tangentially. Following full bloom the cells of the entire inner epidermis, except for narrow longitudinal strips in the region of the dorsal and ventral bundles, become rapidly further elongate. The direction of elongation is oblique, apically toward the dorsal carpellary bundle. Although they are similar to the cells of the

cartilaginous pericarp, they are slightly larger. At maturity they measure 150 μ radially by 12 μ tangentially in cross-section. They may attain a length of 185 μ.

The epidermal cells in the regions of the dorsal and ventral bundles undergo no such elongation but retain nearly isodiametric dimensions, except for occasional large and irregular cells seen here and there where adjacent carpels abut. During the second and third months after full bloom, and apparently continuing until fruit ripening, the cell walls become greatly thickened, until at fruit ripening the lumen is scarcely more than a line (figs. 34–39).

The cartilaginous pericarp immediately underlies the inner epidermis (figs. 34–39). It is derived from the layer of cells immediately beneath the inner epidermis by rapid cell division just before and just after full bloom. Whether this layer is in turn derived from the inner epidermis by cell division several weeks before full bloom, however, could not be exactly determined. From such examples of cell division as figures 30 and 35, and from the behavior of similar tissues in the cherry and related fruits, it would not be surprising if this were the case.

At full bloom the cells measure about 6×6 μ in cross-section. Immediately after full bloom they become steadily more elongate, much as the cells of the inner epidermis, until they may reach a maximum length of 185 μ by 58 days after full bloom. Cell division in radial direction is apparently completed by 25 days after full bloom, for the number of layers of cells comprising this tissue remains at five or six after that time. Dur-

TABLE 1

DIAMETER OF CELLS (μ) OF DIFFERENT TISSUES OF APPLE FRUIT

Tissue	April 19	May 14	June 11	July 11	August 6	September 11
Outer epidermis.	11× 6*	20× 8	18×10	14× 18	12– 14× 20	9× 22
Hypodermis.....	11×11	11×18	11×23	16× 32	14– 16× 36	12× 40– 54
Cortex.....	11× 9	17×21	57×62	106×100	110×120	174–264×197–340
Pith.....	14×13	22×22	71×57	150×110	190×120	314–444×165–268
Fleshy pericarp..	11×10	14×15	36×22	80× 74	90–102× 80–90	169–310× 97–155
Cartilaginous pericarp.....	10× 7	18× 8	63– 94× 8	63–185× 8	63–185× 8	63–185× 8
Inner epidermis..	10× 5	20× 8	130×12	130–174× 12	130–174× 12	130–174× 12

* Radial×tangential.

TABLE 2

NUMBER OF LAYERS OF CELLS IN DIFFERENT TISSUES OF APPLE FRUIT

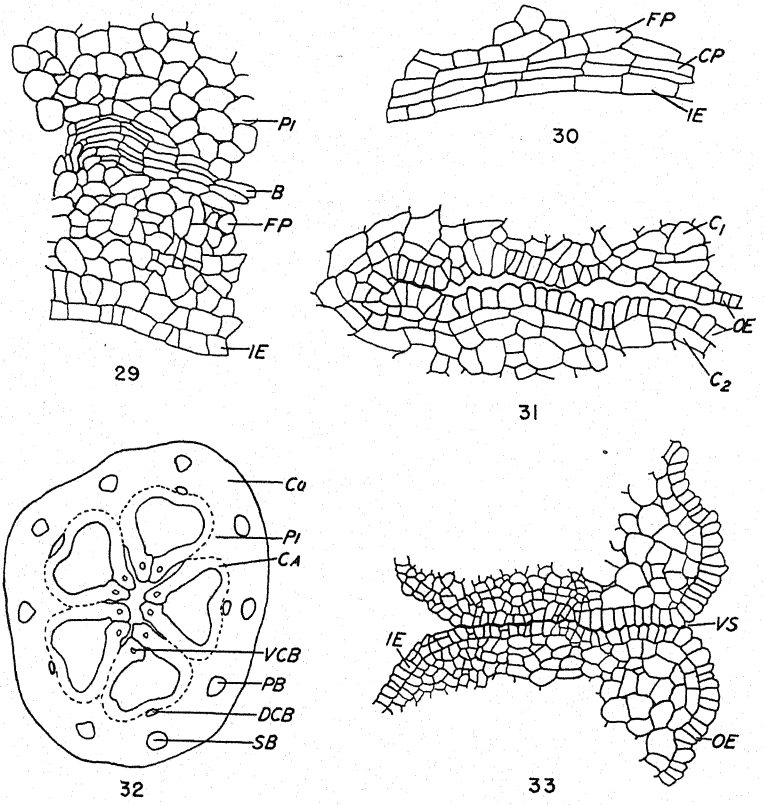
Tissue	April 19	May 14	May 25	May 30	June 11	July 11	August 6	September 11
Outer epidermis.....	1	1	1	1	1	1	1	1
Cortex*.....	17	33	33	38	65	68–70	68–70	68–70
Pith†.....	10	12–13	25	28	35	35–37	35–37	35–37
Fleshy pericarp wall.....	6–8	7	8–9	9–10	10–11	12	12	12
Cartilaginous pericarp wall and inner epidermis.....			3	4–5	5–6	5–6	5–6	5–6

* From outer epidermis to sepal-petal bundles.

† From sepal-petal bundles to dorsal carpellary bundle.

ing the second month after full bloom the cell walls begin rapid thickening, which progresses more slowly during the third and fourth months, until at fruit ripening the lumen is about one-fifth the diameter of the cells.

surrounding these bundles is similar to the condition in the sour cherry already described (27) but much more marked. In fact, in the varieties here observed the hardened portions of the pericarp form two disconnected sheets of tissue, one on



FIGS. 29–33.—Miscellaneous carpel drawings of Twenty Ounce apple at time of full bloom: Fig. 29, cross-section of carpel and portion of pith, in vicinity of ventral carpellary bundle: *pi*, pith; *b*, line of demarcation between pith and carpel; *fp*, fleshy pericarp; *ie*, inner epidermis. Fig. 30, cross-section of carpel showing cell division and origin of tissues: *fp*, fleshy pericarp; *cp*, cartilaginous pericarp; *ie*, inner epidermis. Fig. 31, cross-section of region of two adjacent carpels: c_1, carpel 1; c_2, carpel 2; *oe*, outer epidermis. Fig. 32, cross-section of fruit at stage at which carpel outline appears sharply defined: *co*, cortex; *pi*, pith; *ca*, carpel; *vcb*, ventral carpellary bundle; *pb*, petal bundle; *dcb*, dorsal carpellary bundle; *sb*, sepal bundle. Fig. 33, cross-section through ventral suture of single carpel: *vs*, ventral suture; *oe*, outer epidermis; *ie*, inner epidermis.

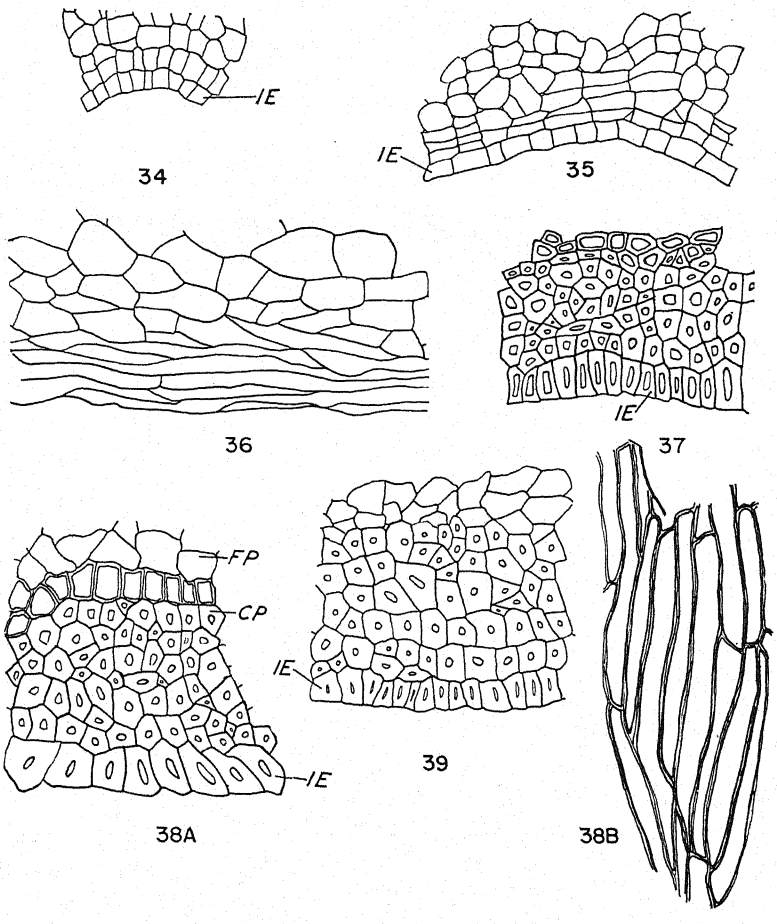
The cartilaginous tissue is five to six cells thick in certain areas but becomes progressively thinner and finally absent toward the regions of the ventral and dorsal carpellary bundles. The absence of sclerenchymatous cells in the tissues

either side of the locule, extending from the proximal to the distal end of the carpel blade.

As the fruit nears ripening, the cartilaginous pericarp may become fissured or split, presumably owing to continued

growth of surrounding tissue. In some varieties, as Twenty Ounce, cells of the fleshy pericarp may become active, so that a callus-like tissue grows out between the breaks in the cartilaginous lining. This brings about the tufted condition noted by horticulturists as varietal characteristics. The splitting of the wall may result in an increase in the gross length or breadth of the cartilaginous carpel and explains how the measurements for such a hard tissue may show an increase (figs. 3, 5–28, 69).

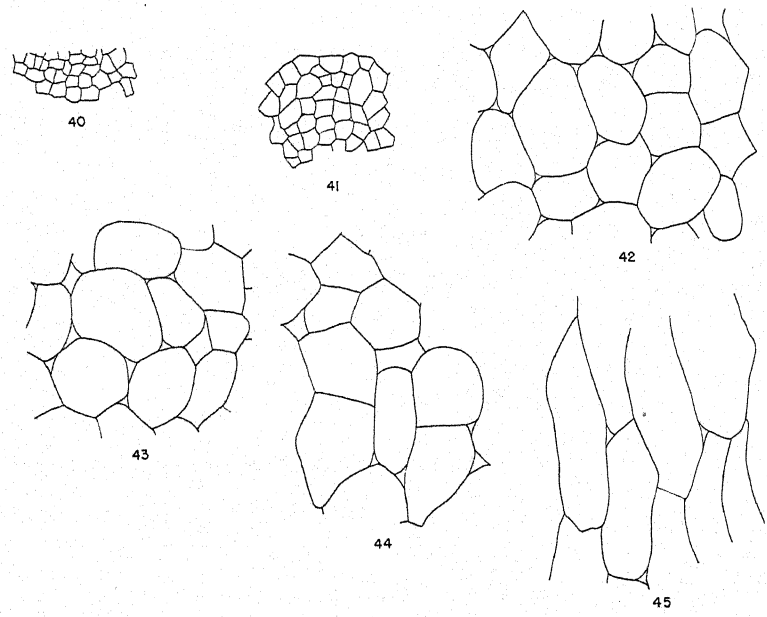
The fleshy pericarp lies immediately adjacent to the cartilaginous pericarp, by which its inner surface is largely bounded. For a short distance near the dorsal carpellary bundle, where the sheets of cartilaginous tissue do not quite touch, it is bounded by inner epidermis, while for a short distance near the region of the ventral carpellary bundles it is bounded by a well-defined outer epidermis (figs. 31, 33). The remainder of the tissue is intimately associated with the adjacent cells of the pith but can be



FIGS. 34–39.—Cellular changes in cartilaginous pericarp of McIntosh apple: Fig. 34, 1 month before full bloom; fig. 35, full bloom; fig. 36, 1 month after full bloom, longisection showing elongation of cells; fig. 37, 2 months after full bloom; fig. 38*A*, 3 months; fig. 38*B*, 3 months, longisection showing elongation of cells; fig. 39, 4 months. *ie*, inner epidermis; *cp*, cartilaginous pericarp; *fp*, fleshy pericarp.

easily recognized as a distinct tissue, even with the unaided eye, by lighter color and greater density (fig. 32). The fine vascular bundles which traverse it are especially conspicuous near the periphery and occasionally sharply separate it from the pith (fig. 29). As shown by KRAUS (12), the thickness of the tissue varies greatly with the variety. Early Harvest and Twenty Ounce have a large fleshy pericarp while in McIntosh it is very small in extent, being only twelve layers of cells thick at its greatest.

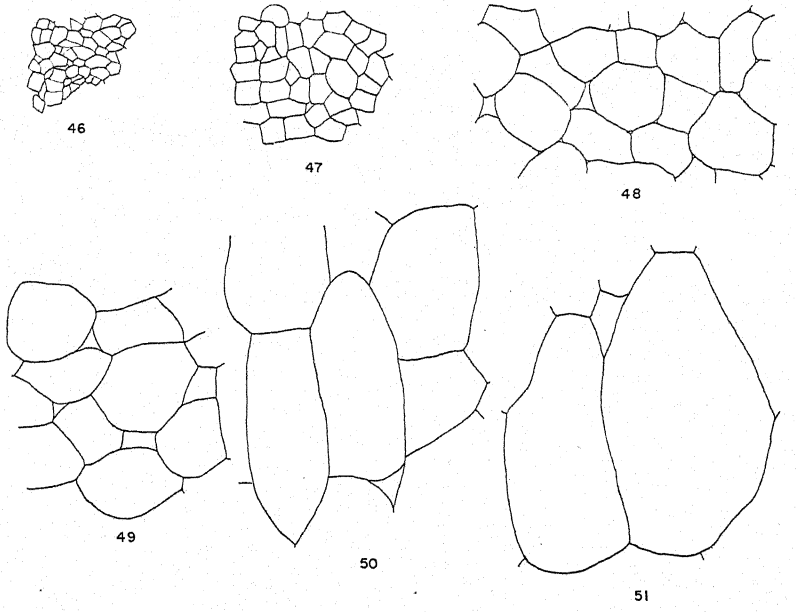
One month before full bloom it is not possible to distinguish the cartilaginous portion of the pericarp from the fleshy portion. The entire carpel wall is at that time made up of seven to nine layers of nearly isodiametric cells, about 10 μ in diameter, except for elongate cells in the peripheral layers which later become differentiated as spiral tracheids (figs. 40–45). By full bloom the portion of the carpel which is to give rise to cartilaginous tissue can be separated from the portion that is to remain fleshy by the tangential division and elongation of those cells. In the fleshy pericarp the cells increase rapidly in size for the first 50 days following full bloom but remain isodiametric. In the region of the ventral suture they remain so until fruit ripening, but throughout most of the carpel wall the parenchymatous cells become greatly elongate in a radial direction later in the season, very much after the manner of cells in the inner portion of the fleshy pericarp of drupe fruits (27). At fruit ripening these cells may average 125 μ tangentially by 300 μ radially. Intercellular spaces are relatively large and numerous.



FIGS. 40–45.—Cellular changes in fleshy pericarp of McIntosh apple from 1 month before full bloom to fruit ripening: Fig. 40, 1 month before full bloom; fig. 41, full bloom; fig. 42, 1 month after full bloom; fig. 43, 2 months; fig. 44, 3 months; fig. 45, 4 months (ripe).

PITH.—The pith is bounded on its inner surface by the fleshy pericarp of the

carpels and on its outer surface by the cortex. The core line lies between the cortex and the pith and is variable in outline for different varieties (**11, 13**). In early stages of development, particularly before full bloom, it is difficult to distinguish this region. In cross-sections of McIntosh cut 1 week after full bloom, about 18 μ. Intercellular spaces become prominent and remain so until fruit ripening. For a period of 11 days just at full bloom and immediately following there is much cell division, the number of layers just about doubling. For the next 17 days there is a less active period of cell division, until the maximum num-



FIGS. 46–51.—Cellular changes in pith of McIntosh apple from 1 month before full bloom to fruit ripening: Fig. 46, 1 month before full bloom; fig. 47, full bloom; fig. 48, 1 month after full bloom; fig. 49, 2 months; fig. 50, 3 months; fig. 51, 4 months (ripe).

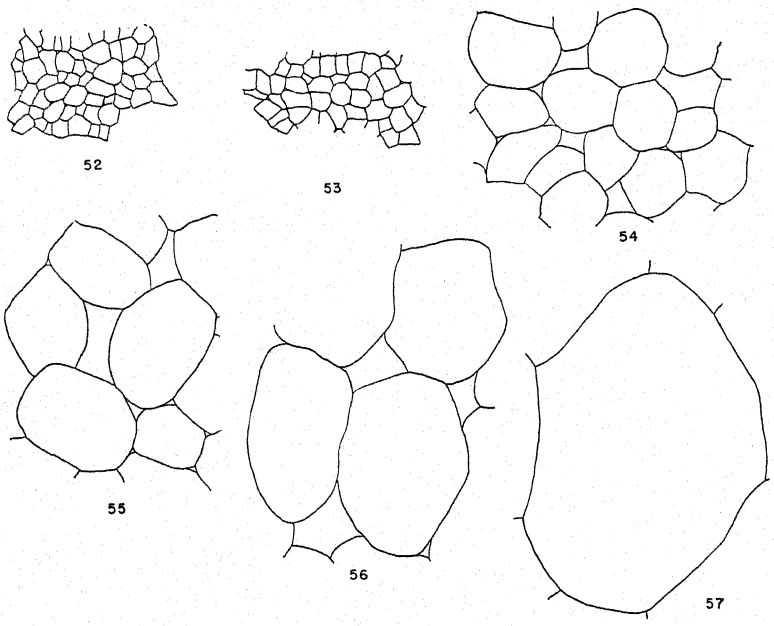
the core line appears as a tissue composed of isodiametric cells larger than either the pith or cortex. In later stages, however, these cells do not increase in size as markedly as those of the cortex and pith, so that they constitute a region of smaller tangentially elongate cells (**17**).

The pith can be recognized as a distinct tissue in material fixed as early as 1 month before full bloom. At that time the cells are isodiametric and about 14 μ in diameter (figs. 46–51). Intercellular spaces are small and not conspicuous. By full bloom the cells have increased to ber of layers of cells—thirty-five to thirty-seven—is reached, although this number may vary with season, variety, and size of fruit (**19**).

As fruit development proceeds, the cells increase greatly in size (especially during the third and fourth months after full bloom), become radially elongate, and finally attain average dimensions of about 150×300 μ. Intercellular spores are prominent and conspicuous and become progressively larger, greater increase occurring during the last month preceding fruit maturity. Since cell divi-

sions appear to have ceased in the pith by about 3 weeks after full bloom, increase in size of this tissue is due thereafter solely to increase in size of cells and of intercellular spaces.

CORTEX.—The cortex 1 month before full bloom is composed of about seventeen layers of isodiametric cells, with an average diameter of about 10 μ (figs. 52–57). At this stage the cortical vascular network is discernible but only partially differentiated. Although the cells increase in size from this time to full bloom, the significant feature of the period is the rapid increase in the number of cells, so that there are about thirty-three layers at full bloom, although this number may vary with season, variety, and size of fruit (19). Intercellular spaces are small and inconspicuous. During the next 2 weeks there is both rapid increase in cell size and also increase in number of cells, so that by that time there are sixty-five layers of cells about 60 μ in diameter. Cell division seems to be about completed in this tissue by 3 weeks after full bloom for the variety McIntosh. MACARTHUR and WETMORE (16) report general cell division completed before the end of June. All subsequent increase is by enlargement of both cells and intercellular spaces. Cells in the central region of the cortex are radially elongate and may attain the relatively great size of 197×340 μ. Those nearer the periphery are less elongate, isodiametric, or even slightly tangentially elongate immediately beneath the hypodermal layer, suggesting the condition observed in the cherry (27). Intercellular spaces increase greatly during the 2 months preceding fruit maturity and are a conspicuous feature of the thin-walled cortical tissue.



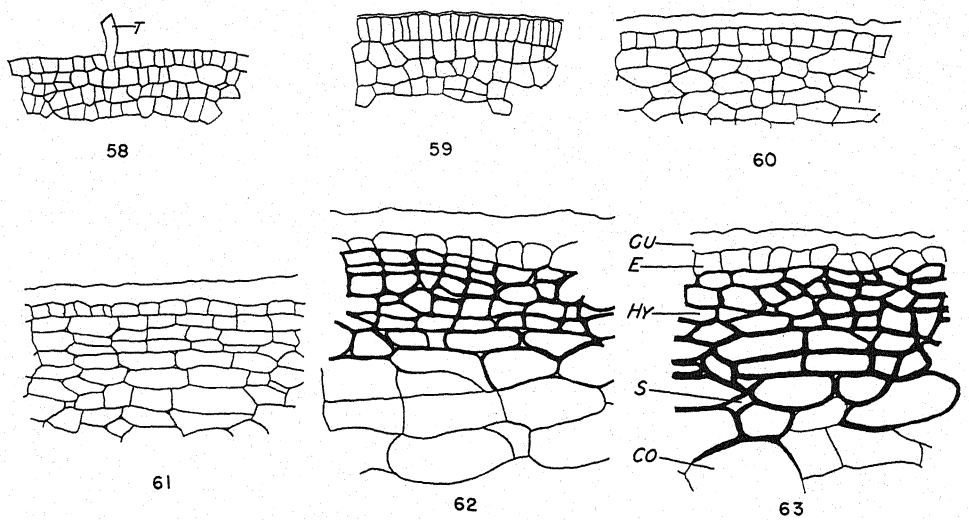
FIGS. 52–57.—Cellular changes in cortex of McIntosh apple from 1 month before full bloom to fruit ripening: Fig. 52, 1 month before full bloom; fig. 53, full bloom; fig. 54, 1 month after full bloom; fig. 55, 2 months; fig. 56, 3 months; fig. 57, 4 months (ripe).

HYPODERMAL LAYER.—The hypoder-

mal layer is part of the cortex immediately beneath the outer epidermis, characterized in the mature fruit by thick-walled collenchyma in which the protoplastic contents have become granular in nature and are less densely pigmented (figs. 58–63). Together with the epidermis it forms the skin of the fruit. One month before full bloom it cannot be distinguished from the adjacent cells of the cortex, being composed of thin-walled isodiametric cells about 11 μ in diameter. By full bloom the walls of the first row or two of cells immediately below the epidermis have begun to thicken and have increased slightly tangentially. They are, however, smaller than the cells of the adjacent cortex and easily defined as a distinct tissue. Both tangential elongation and thickening of the walls continue until the fruit has attained full size. At maturity the cells are scarcely larger in radial diameter than they were 1 month before full bloom but have increased tangentially four or five fold. In the course of development they are further crowded and stretched, so that any semblance of regularity observed in earlier stages becomes lost. It is often difficult to determine the limits of an individual cell or to decide whether indefinite areas are crushed cells or crushed air spaces, as described by BELL (3, 4).

EPIDERMIS.—The epidermis is a uniseriate layer of palisade-like cells which can be identified even 1 month before full bloom (figs. 58–63). At that time the cells are radially elongate, being about 6 μ in tangential diameter and 11 μ in radial diameter. Together with the hypodermal layer it forms the skin of the fruit.

Epidermal hairs are sufficiently numerous to form a dense pubescence. BELL (3) has traced in detail the separation of trichomes from one another by the division of the epidermal cells which lie between them. He has also shown the



FIGS. 58–63.—Cellular changes in epidermis and hypodermal layer of McIntosh apple from 1 month before full bloom to fruit ripening: Fig. 58, 1 month before full bloom; fig. 59, full bloom; fig. 60, 1 month after full bloom; fig. 61, 2 months; fig. 62, 3 months; fig. 63, 4 months (ripe). *t*, trichome; *cu*, cuticle; *e*, epidermis; *hy*, hypodermal layer; *s*, intercellular space; *co*, cortex.

manner in which the trichomes are finally eliminated by development of the cuticle beneath their bases.

By full bloom the cells are still further radially elongate, 20×8 μ, and are slightly thickened. The cuticle appears in cross-section as scarcely more than a line. From this time on, the cells become more tangentially elongate, until at fruit ripening they are actually slightly smaller in radial diameter than they were 1 month before full bloom and two and one-half to three times as large in tangential diameter, 9×22 μ. They remain relatively thin walled.

According to BELL (3), cell division ceases in the variety McIntosh in Nova Scotia during the middle of June, when full bloom was June 2. TETLEY (22) reports division in Bramley Seedling on June 12 in material collected near Cambridge, England.

Since it is difficult to be certain when cell division has been completed in tissue by a search for mitotic figures, the writers resorted to counting the epidermal cells in a quadrant of a cross-section of the fruit at varying intervals, from 1 month prior to full bloom until fruit ripening, for both McIntosh and Twenty Ounce, and multiplying this figure by four as though the apple were a perfect sphere. The figures are given in table 3, together with the transverse diameters of the cells during the period. From these figures it will be seen that the number of epidermal cells in the circumference of a cross-section is just slightly more than doubled from the twenty-fifth to the third day before full bloom. From the third day before full bloom until the twenty-fourth day after, it is increased more than six times for McIntosh and more than three times for Twenty Ounce. From then on the increase in number is not great but seems to continue, even 85 days after full bloom. It will be observed further that during the period when cell divisions are most rapid the increase in the tangential diameter is not great; but later in the season, when cell divisions are few if any, the tangential diameter markedly increases to accommodate the increasing surface area.

TABLE 3

NUMBER AND SIZE OF CELLS OF OUTER EPIDERMIS

DATE*	TRANSVERSE DIAMETER OF CELLS OF OUTER EPIDERMIS (μ)	NO. OF CELLS IN CIRCUMFERENCE OF CROSS-SECTION	
		MCINTOSH	TWENTY OUNCE
April 19	.....	500	932
May 14	.....	1124	1960
20	8.9	.....	.....
27	10.2	.....	.....
June 3	11.3	.....	.....
10	12.7	7500	7852
27	12.8	.....	.....
28	13.5	.....	.....
July 2	14.2	.....	.....
12	15.5	8388	9276
27	16.3	.....	.....
Aug. 10	21.8	8600	9716
Sept. 11	23.8	9272	.....
28	.....	.....	9252

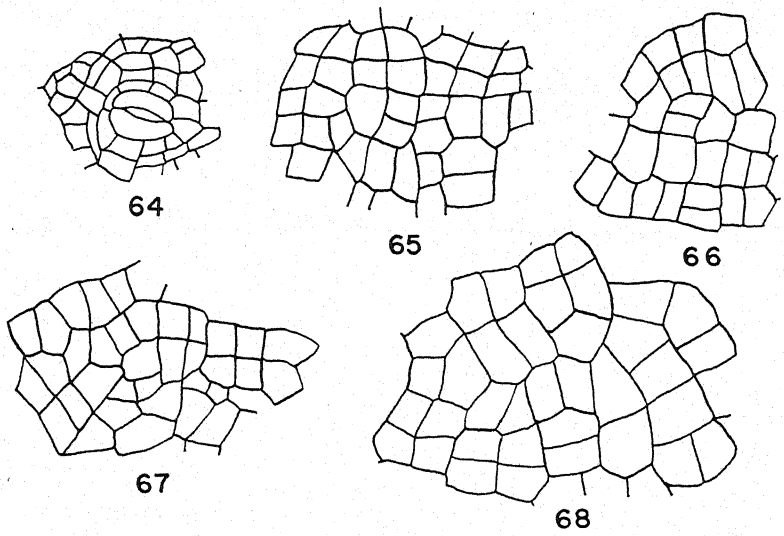
* Full bloom May 17.

As a final check on the accuracy of the measurements, average diameters of the cells were multiplied by the number counted at varying intervals. The resulting figures approximate very closely the circumference of the fruit at those times.

Obviously such a measure, because of variation in samplings and other factors, cannot be an absolute record of the exact termination of cell division, but it does indicate that although general cell division throughout the fruit is completed fairly early in fruit development, yet cell division in the epidermis continues later at Geneva, New York, for both Mc-

Intosh and Twenty Ounce than has previously been recorded in the literature.

Figures 64–68 show the epidermis in section tangential to the surface at monthly intervals from full bloom to fruit ripening. The drawings were made by stripping the epidermis from the fruit, mounting and viewing by transmitted light, focusing upon the narrowest portion of the cell wall. If viewed in surface, walls appear much thicker, as shown by BELL (3) and TETLEY (21), owing to the greater thickening of the cells at the corners and the crowned shape which many of them assume. It is interesting to observe the apparent pairing of the cells and even the quadrant appearance.



FIGS. 64–68.—Cells of epidermis in surface view from full bloom to fruit ripening. Note evidences of cell division.

Relation between development of embryo, nucellus and integuments, carpel, and entire fruit

In the peach and cherry (23–26) there is an interesting relationship between development of the embryo, nucellus and integuments, and fruit. The fruit develops in three clearly defined stages: stage I, rapid increase for a period immediately following full bloom; stage II, retarded development for a period during mid-season; and stage III, renewed rapid increase to fruit ripening. The nucellus and integuments reach full size during stage I, while the embryo remains microscopic. During stage II the stony pericarp hardens and the embryo makes rapid increase.

As will be seen in figure 69, there are no such stages of growth in the apple fruit as measured by the over-all length. This might be expected, however, since the apple is an accessory fruit whose bulk is stem tissue. Within the stem portion, however, the carpels behave somewhat similar to those of the drupe fruits mentioned. The carpel blade reaches full length relatively early, likewise the nucellus and integuments, while the embryo remains microscopic for a considerable period (7, 20) after full bloom.

Further, the curves of growth for the three varieties (Early Harvest, McIntosh, and Rome) are similar for development of the embryo and nucellus and integuments, regardless of the fact that early, mid-season, and late-ripening varieties are represented.

course, as has been possible with the cherry and peach. Instead, the curves for the late-ripening Rome are somewhat less steep, particularly for development of the cartilaginous portion of the carpel.

The writers have the impression, however, that there is greater similarity between development of the embryo, nucellus and integuments, and carpel of the apple and of the drupe fruits mentioned than these figures show. Part of the difficulty is to secure samplings of the apple as uniform as possible with those of the peach and cherry. Fertilization occurs over a greater period of time with

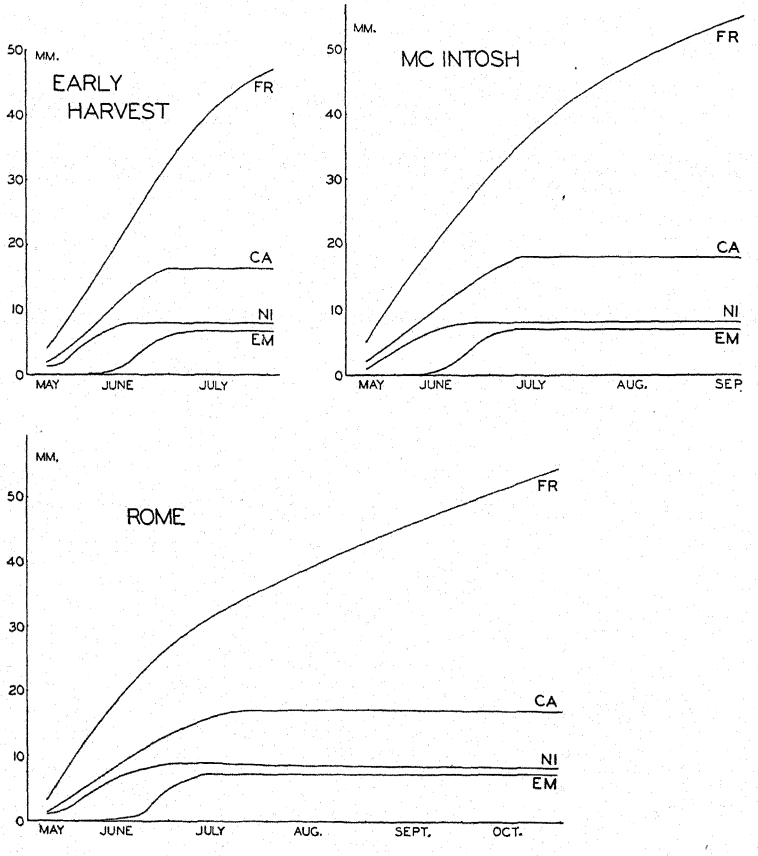


FIG. 69.—Increase in length of embryo (*em*), nucellus and integuments (*ni*), carpel (*ca*), and entire fruit (*fr*) of Early Harvest, McIntosh, and Rome apples from full bloom to fruit ripening.

Here the similarity ends, however, because the embryo apparently begins rapid development slightly before the nucellus and integuments have reached full length, and the nucellus and integuments in turn have reached maximum size before the carpel blade has reached maximum length. Further, the curves cannot be interposed over their entire

the apple than with the cherry and plum, development of fruits from central and lateral blossoms in the apple inflorescence is different, and the number of seeds in a carpel markedly affects development. The resulting variability masks some of the finer details of development.

Summary

1. Gross development and principal tissue changes in the fruit of the apple from 1 month before full bloom throughout the growing season to fruit ripening are figured and discussed, and suggestions are made regarding the methods and the values of measurements of various parts.

2. Interpretation of the apple as five drupelike carpels contained within the fleshy torus or receptacle has seemed to describe the structures observed.

3. The curve of gross development of the entire fruit is nearly a straight line for the early summer variety (Early Harvest), but for each successively later-ripening variety (McIntosh and Rome) the curve flattens as the season progresses. Although perhaps associated with environment, the rate of growth is shown to be a varietal characteristic, in which each successively later-ripening variety has a slower rate.

4. The cartilaginous portion of the carpels develops rapidly for 2–4 weeks after full bloom, reaching maximum size in transverse diameter the earliest of any of the tissues making up the bulk of the fruit. The fleshy portion of the carpels continues growth approximately 2 weeks longer and is the next tissue to reach maximum size. The pith and cortical regions continue growth up to fruit ripening and constitute the bulk of the apple at that time.

5. The edges of the carpels are in approximate contact along the ventral suture up to about 4 weeks after full bloom. In such varieties as the Twenty Ounce, the edges then begin to spread apart along the ventral suture and curve away from the central axis of the fruit, resulting in an "open core" condition.

6. Development of the carpels resembles somewhat development of a drupe fruit. Each carpel is composed of inner epidermis, cartilaginous pericarp, fleshy pericarp, and outer epidermis.

7. The cells of the inner epidermis are radially elongate 4 weeks prior to full bloom. Following full bloom the cells of the inner epidermis elongate rapidly obliquely toward the apex of the fruit and toward the dorsal carpellary bundles, except for some longitudinal strips in the region of the dorsal and ventral bundles. During the second and third months after full bloom the cell walls become greatly thickened, until at fruit ripening the lumen is scarcely more than a line.

8. The cartilaginous pericarp increases by cell division prior to full bloom and by cell elongation and some cell division after full bloom. During the second month after full bloom the cell walls begin rapid thickening, which progresses until at fruit ripening the lumen is about one-fifth the diameter of the cells. There is an absence of sclerenchymatous cells in the region of the carpel bundles, so that the hardened portions of the pericarp form two disconnected sheets of tissue, one on either side of the locule. As the fruit nears ripening the cartilaginous pericarp may become fissured or split, resulting in a tufted condition when parenchyma tissue grows between the breaks. The gross size of the cartilaginous carpel may increase by this means, even after hardening is complete.

9. The fleshy pericarp may be distinguished from the cartilaginous pericarp at full bloom. Cell division is most

abundant prior to and at the time of full bloom. Cell enlargement follows, many of the cells becoming radially elongate and reaching 300 μ at fruit ripening, as compared with 10 μ at full bloom.

10. The pith can be recognized as early as 1 month before full bloom. There is much cell division for 11 days just at and immediately after full bloom. Cell division seems to have ceased by 3 weeks after full bloom. Increase in size of pith is due thereafter to increase in size of cells and of intercellular spaces, some cells reaching 150×300 μ.

11. The cortical region prior to full bloom increases rapidly in number of cells, by cell division. During the next 2 weeks there is rapid increase in both number and size. Cell division appears complete 3 weeks after full bloom, so that subsequent increase in size is by enlargement of cells and of intercellular spaces, some cells attaining a size of 197×340 μ.

12. Cells of the hypodermal layer can be distinguished by full bloom by slight thickening of the walls. Both tangential elongation and thickening of the walls continue until the fruit has attained full size.

13. The cells of the epidermis are palisade-like and readily distinguished at least 1 month before full bloom. The number of cells is more than doubled between the twenty-fifth and the third day before full bloom. From the third day before full bloom until the twenty-fourth day after it increases more than six times and appears to continue to some degree until 85 days after full bloom. As the period of fruit ripening approaches there is much tangential stretching of the cells to accommodate the increasing surface area.

14. Development of pericarp, nucellus and integuments, and embryo is similar in some details to the stages of development of similar parts in drupe fruits; but the bulk of the apple fruit, being accessory tissue, shows no such stages.

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LITERATURE CITED

1. ARBER, AGNES, The interpretation of the flower: a study of some aspects of morphological thought. Biol. Rev. 12:157–184. 1937.
2. ASKEW, H. O., Changes in the chemical composition of developing apples. Jour. Pom. and Hort. Sci. 13:232–246. 1935.
3. BELL, H. P., The protective layers of the apple. Can. Jour. Res. 15:391–402. 1937.
4. ———, The origin of russeting in the Golden Russet apple. Can. Jour. Res. 15:560–566. 1937.
5. BLACK, CAROLINE A., The nature of the inflorescence and fruit of *Pyrus malus*. Mem. New York Bot. Gard. 6:519–547. 1916.
6. CLEMENTS, H. F., Morphology and physiology of the pome lenticels of *Pyrus malus*. BOT. GAZ. 97:101–117. 1935.
7. ELSSMANN, E., and VON VEH, R., Beiträge zur Frage nach den Befruchtungsverhältnissen der für Deutschland wirtschaftlich wertvollsten Kern-, Stein- und Beerenobstsorten I. Gartenbauwiss. 6:1–54. 1931.
8. EWERT, RICHARD, Der Einfluss der Kernes auf die Entwicklung der Frucht und auf die Ernährungsvorgänge in Baume. Jahrb. Königl. Pom. Inst. Proskau 1908:95. 1910.
9. ———, Die Abhängigkeit des Reiseprozesses der Frucht von der Entwicklung der Samen. Jahrb. Königl. Pom. Inst. Proskau 1909:110–111. 1910.
10. FARMER, J. B., Contributions to the morphology and physiology of pulpy fruits. Ann. Bot. 3: 393–414. 1889.
11. KRAUS, E. J., The pollination of the pomaceous fruits. I. Gross morphology of the apple. Oregon Agr. Exp. Sta. Res. Bull. 1. 1913.
12. ———, Variation of the internal structure of apple varieties. Oregon Agr. Coll. Exp. Sta. Bull. 135. 1916.
13. KRAUS, E. J., and RALSTON, G. S., The pollination of the pomaceous fruits. III. Gross vascular anatomy of the apple. Oregon Agr. Coll. Exp. Sta. Bull. 138. 1916.
14. LAMPE, PAUL, Zur Kenntniss des Baues und der

Entwickelung saftiger Früchte. Zeitschr. Naturwiss. **59**:294–323. 1886.

15. MACARTHUR, MARY, and WETMORE, R. H., Developmental studies in the apple fruit in the varieties McIntosh Red and Wagener. I. Vascular anatomy. Jour. Pom. and Hort. Sci. **17**: 218–232. 1939.
16. ———, and ———, Developmental studies of the apple fruit in the varieties McIntosh Red and Wagener. II. An analysis of development. Can. Jour. Bot. **19**:371–382. 1941.
17. MACDANIELS, L. H., The morphology of the apple and other pome fruits. Cornell Agr. Exp. Sta. Mem. 230. 1940.
18. SEN, P. K., Some observations on the time of development of the fruit, seed and embryo in apple. Ann. Rpt. East Malling Res. Sta. **1936**: 137–141. 1937.
19. SMITH, W. H., The histological structure of the flesh of the apple in relation to growth and senescence. Jour. Pom. and Hort. Sci. **18**:249–260. 1940.
20. STEINEGGER, PAUL, Zytologisch bedingte Ei- und Zygotensterilität bei triploiden Apfelsorten. Ber. Schwiz. Bot. Ges. **42**:285–339. 1933.
21. TETLEY, U., A study of the anatomical development of the apple and some observations on the "pectic constituents" of the cell walls. Jour. Pom. and Hort. Sci. **8**:153–172. 1930.
22. ———, The morphology and cytology of the apple fruit, with special reference to the Bramley's seedling variety. Jour. Pom. and Hort. Sci. **9**:278–297. 1931.
23. TUKEY, H. B., Embryo abortion in early ripening varieties of *Prunus avium*. BOT. GAZ. **44**: 433–468. 1933.
24. ———, Growth of the peach embryo in relation to growth of fruit and season of ripening. Proc. Amer. Soc. Hort. Sci. **30**:209–218. 1933.
25. ———, Growth of the embryo, seed, and pericarp of the sour cherry (*Prunus cerasus*) in relation to season of fruit ripening. Proc. Amer. Soc. Hort. Sci. **31**:125–144. 1934.
26. ———, Development of cherry and peach fruits as affected by destruction of the embryo. BOT. GAZ. **98**:1–24. 1936.
27. TUKEY, H. B., and YOUNG, J. O., Histological study of the developing fruit of the sour cherry. BOT. GAZ. **100**:723–749. 1939.
28. WHITEHOUSE, W. E., A study of variation in apples during the growing season. Oregon Agr. Coll. Exp. Sta. Bull. 134. 1916.

EFFECTS OF GROWTH-REGULATING SUBSTANCES ON SHOOT DEVELOPMENT OF ROSES DURING COMMON STORAGE

PAUL C. MARTH[1]

(WITH FOURTEEN FIGURES)

Introduction

Roses constitute a large percentage of all nursery stock that is stored, and usually the plants are stored in common storage houses. Such houses do not have mechanical means of refrigeration but depend upon a ventilating system to exchange outside air of low temperature with the warm air within the house. Under these storage conditions rose bushes present a problem to the nurseryman, since their dormancy apparently is more readily broken than that of other nursery material by brief exposures to high temperature. In some years, when it is difficult to lower the temperature within such houses in early winter, the bushes start shoot growth in December, making them difficult to handle properly throughout the storage period. If high outside temperatures occur in late winter, the temperature within the storage house also rises and thereby shortens the period that the bushes can be held safely without excessive shoot production taking place. Spring temperatures are also of considerable influence.

The etiolated shoots that develop in common storage are objectionable from two standpoints. First, they must be removed from the plants soon after they develop, since they are very susceptible to attacks by molds and other organisms that infect the tender new shoots and invade the older stems, ultimately destroying most of the plant tops. Second, much of the stored food reserves are used up in producing the etiolated shoots and consequently are lost from the plants upon their removal.

The discovery in recent years of the inhibiting effect of certain chemical compounds on bud growth when applied to plants suggested the possibility that these materials might be used to prevent shoot growth on rose bushes while in storage. The experiments reported here were undertaken to investigate this possibility.

Material and methods

A number of compounds, in a range of concentrations, as well as various solvents, penetrating agents, and carriers for applying the substances were tested in exploratory trials during the 1939–1940 storage season, using 1200 rose bushes of the Ami Quinard variety. These plants were in lots of three to five each. The preliminary trials indicated that development of vegetative buds could be inhibited, and certain chemical compounds were selected for further trial in the following storage season.

In 1940–1941 the same variety was again employed, using twenty plants in each treatment, a total of 3200 plants. They were all field-grown, no. 1 grade, 1-year-old bushes obtained from a Texas nursery. Unless otherwise indicated, in

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this season all treatments were applied to the dormant stems only, and following treatment the plants were held in common storage for a period of 1–2 months. Previous to treatment the plants in all experimental lots were placed upright in ½-bushel baskets and kept with moist peat packed around the entire root system.

The seventeen compounds used are listed in table 1. Many of these substances are but slightly soluble in water. In preliminary trials all were used at their maximum solubility in aqueous solution sprayed on dormant plants. Although several compounds showed a noticeable effect in delaying bud breaking when supplied in this way, adequate suppression of growth was not obtained with any of them.

Other solvents and carriers were then tried, such as varying percentages (5 to 20 per cent) of ethyl alcohol, dioxan, acetone, lanolin emulsion; 0.25 and 0.125 per cent light oil emulsion, as well as two proprietary wax emulsions frequently used in waxing rose plants commercially. Of these carriers the wax emulsions proved the most satisfactory, for not only could the growth substance be used in higher concentration than in water alone, but the emulsion appeared to be less injurious to the plants than the alcohols and other solvents used. JONES and RICHEY (6) have reported that wax emulsions could be applied with safety to young tomato plants. In spraying large lots of plants, a small, electrically operated, pint-capacity paint sprayer was used. This type of sprayer produced a smooth uniform film with better spray coverage than that obtained with several types of hand sprayers tested.

The esters of naphthaleneacetic acid (naphthalenemethylacetate and naphthalenethylacetate) volatilize readily on heating, and their fumes were used in treating plants by the vapor method. Another compound, naphthaleneacetonitrile, was used in the same manner.

In applying the gases it was found essential to have the rooms or containers airtight. It was also necessary to provide heating equipment within such rooms or containers to volatilize the liquid growth substances and a fan to develop equal distribution throughout the chamber. Thus, treatments were applied in a common nursery storage room of 28,000 cubic feet and in a small stockroom of 1000 cubic feet, with openings sealed with putty; in 20-gallon galvanized cans having tight-fitting lids sealed with adhesive tape; in a double-thickness paper-lined tongue-and-grooved wooden chamber of 400 cubic feet, used for cyanide fumigation; in large bell jars with ground-glass bottoms and 2-inch diameter openings in the top; also in the chamber illustrated in figure 1, constructed of two 50-gallon metal drums welded end-to-end and with a water-sealed cover.

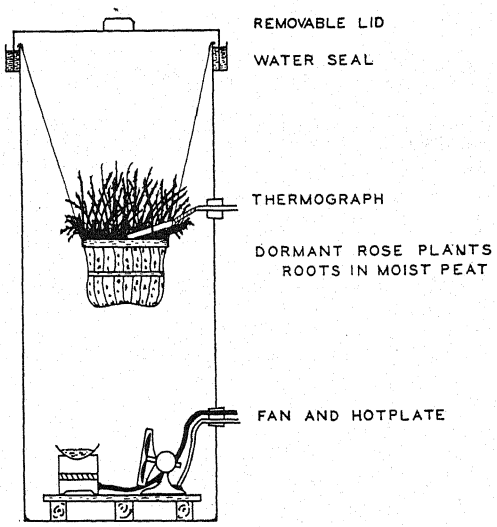


FIG. 1.—Apparatus used in treating rose bushes with volatilized growth substance under controlled conditions.

In all cases the exact capacity of the chamber was known, so that the concentration of growth substance could be calculated for a comparable chamber of 1000 cubic feet capacity. In the very small chambers, employing low concentration of gas, a readily measurable weight of the substance was first dissolved in absolute alcohol and the necessary quantity of this solution then added. It was found that, upon heating, the pure substance would sometimes char; but if it were mixed with 95 per cent alcohol and then placed in a shallow watch glass, volatilization would be complete without visible residue. The alcohol also greatly lessened the time required for volatilization, which was of considerable aid in reducing the amount of heat required in the process. In treatments where it was necessary to have a controlled temperature, especially at the lower temperatures, avoidance of heating was desirable.

As well as alcoholic solutions, attempts were made to volatilize dry crystals of a number of growth substances that occur in crystalline form at room temperature, since ZIMMERMAN and HITCHCOCK (17) have reported that many are active when used in this way. These attempts met with little success. Although most of the substances tried could be volatilized to a greater or less degree, considerable recrystallization and charring took place. Under these circumstances the exact concentration of gaseous growth substance would be difficult to determine and repeat under a given set of conditions. For this reason, with the gaseous applications emphasis was placed on the use of the esters of naphthaleneacetic acid in alcoholic solution. These volatilize with a minimum of heat application and leave no visible residue. This is also true of the naphthaleneacetonitrile compound.

In most of the preliminary experiments in 1939–1940 the plants were moved to the greenhouse immediately after applying the growth substance. Greenhouse forcing was employed in order to speed up the testing of a great number of compounds at varying concentrations as well as the effect of the different carriers. Any delay in shoot development in a given treatment, as compared with similar control plants, was then used as a measure of the effectiveness of the particular treatment on inhibition.

To compare the effectiveness of the various treatments, data on number and length of shoots were taken on an individual-plant basis at weekly intervals during the storage tests. At the same time, record was made also of the length of the injured portions of the canes on each plant.

In order to compare the effect of treatments on dormancy, sections of the stems were examined for starch disappearance by the IKI test. These sections were obtained from the stock portion of a number of plants exhibiting varying stages of cane maturity. Ten sections were chosen to represent a range in relative starch content in ten arbitrary gradations, on a scale from 0 to 100 (fig. 2). The sections were made freehand with a sharp straight-edge razor, from material killed by boiling in 85 per cent alcohol. After staining, the sections were mounted on glass slides in 50-50 alcohol-glycerin, with cover glasses sealed by paraffin to make semi-permanent mounts. The original material from which each of the ten sections was made was held in 85 per cent alcohol and used for new standard sections throughout the period of sampling, so that any changes in the standard could be detected.

Figure 2 shows a camera lucida draw-

ing of all sections except the 100 gradation, since no section was found scoring that high. Scoring for relative starch content was made separately on bark (fig. 2*A*), which included all tissues exterior to the cambium (fig. 2*B*); on wood (fig. 2*C*), composed of xylem tissues from the cambium to the pith; and on the pith (fig. 2*D*). a standard slide was placed on the stage of a microscope side by side with slides containing freehand sections of the material being examined. Each section was thus matched to the nearest corresponding standard, using a magnification of 10. In the beginning twenty sections were made of each plant part, but later so

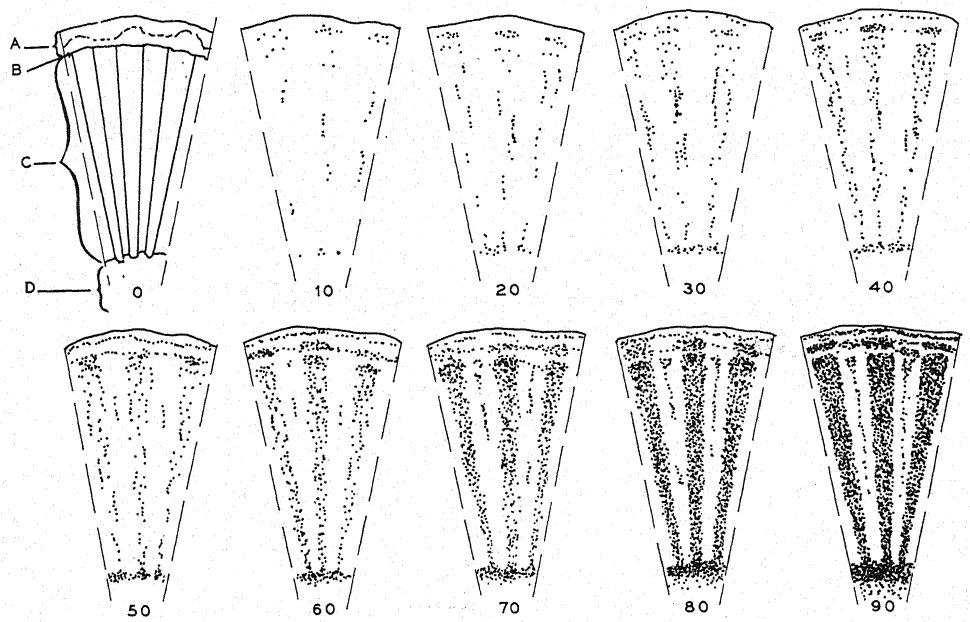


FIG. 2.—Camera lucida drawing of cross-sections of rose stocks used as standards for comparison in estimating relative starch content by the IKI method: *A*, bark and cortex region; *B*, position of cambium in respect to other tissues; *C*, wood; *D*, pith.

A random sample of twenty plants was selected from each lot at each sampling period. Segments about 1 inch long were taken of each plant from the base of the canes, from the mid-portion of the stock, and from the roots at their juncture with the stock. These segments were dropped into separate flasks containing 85 per cent alcohol and boiled for 30 minutes in a waterbath. After cooling, the segments were ready for estimation of relative starch content, which was made on the same day that the samples were taken.

For estimating relative starch content, little variability was found within a particular segment that this number was reduced to ten.

Experimental results

The 1060 bushes used in the principal experiment were separated into fifty-three uniform lots of twenty plants each. Application of each compound at three concentrations, 0.005, 0.01, and 0.05 per cent, respectively, in wax emulsion was used on each lot of twenty plants. Two additional lots of twenty plants each were used for controls, one of which was

sprayed with wax emulsion only and the other left unsprayed. The sprays were applied on February 26, 1941, and the plants were placed in common storage. By April 18, 41 days later, the control lots, as well as many lots treated with growth substance, had produced considerable shoot growth (fig. 3). The number

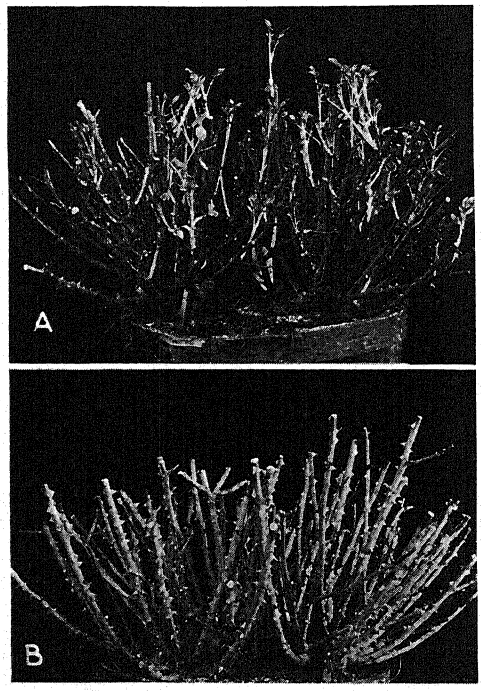


FIG. 3.—Effect of growth-regulating substance on bud inhibition (Ami Quinard): *A*, control; *B*, plants sprayed with wax emulsion of naphthalenemethylacetate. Sprays applied February 26, 1941; plants placed in common storage and photographed April 18.

and length of shoots produced per plant under the different treatments were recorded on this date. After removing all shoots that had developed in storage, the plants were removed from containers and potted in soil in 6-inch pots. The plants were then randomized on a bench in a greenhouse kept at 65°–70° F.

In table 1 the compounds have been arranged in the order of their relative effectiveness in preventing shoot elongation during common air storage. The two esters of naphthaleneacetic acid were more effective than the acid itself. At the 0.005 per cent concentration of the methyl ester complete dormancy was maintained (fig. 3), as was also the case with plants treated at this concentration with the ethyl ester. Both esters were also found to be very effective when applied in the gaseous state, which was also true of naphthaleneacetonitrile. Indoleacetic acid, indolebutyric acid, and naphthalene acetamide, compounds widely reported for their root-promoting effects on cuttings, were about equally effective in preventing shoot growth on the rose plants, with the amide proving superior at the 0.01 per cent concentration; but none of these compounds was as effective as the esters of naphthaleneacetic acid.

Some injury was apparent on all lots treated with 0.05 per cent concentration of every compound except indolepropionic and phenylacetic acids and tetralin-6-acetamide. No injury resulted, even with the highest concentration of each of these three compounds, neither was there any apparent inhibiting effect on shoot development. On the contrary, there was marked and significant increase in the number of vegetative shoots produced by each of these compounds at the lowest concentration (0.005 per cent). Stimulating effects on vegetative shoot production by phenylacetic acid have been noted also on potato tubers (17).

After the plants had been in the greenhouse 14 days, the lots that had received effective growth-substance treatments in common storage continued to show pronounced slowness of development of vegetative buds. This "hold-over" retardation is illustrated by the appearance of the controls and treated lots shown in figure 4.

Data on mean number of shoots produced after 21 days in the greenhouse are shown in table 1. With the great differences found necessary for significance (21.5 at the 5 per cent level), none of the treatments applied in common storage induced significantly greater or less number of shoots after the plants had been in the greenhouse for 21 days following common storage. The extreme variability of these data with regard to number of shoots was unquestionably due to a marked reduction in number of shoots on certain plants owing to strong apical dominance of the uppermost buds, irrespective of prior treatment. These plants produced several strong-growing shoots which appeared to inhibit the remainder of the vegetative buds on their canes. This behavior threw plants with many and plants with few shoots into the same or different lots in an unpredictable manner. It would seem from these data that growth-substance treatments may completely mask individual plant variation as to shoot production in common storage; but that in the greenhouse, after the effect of the treatment has been removed or dissipated, individual plant variability will again express itself by shoot production.

The plants that suffered cane injury from 0.05 per cent growth-substance wax-emulsion sprays in storage (naphthaleneacetonitrile 0.05 per cent treatment)—and therefore had considerable top removed when potted—apparently had sufficient dormant buds at the base of the canes to produce numerous shoots per plant (table 1). The greenhouse conditions of this experiment provided humidity and temperature control of the air as well as control of the soil and its moisture content, within relatively narrow limits. Under these conditions the controls and plants from ineffective storage treatments grew remarkably well. This is in direct contrast with the field-planting results obtained with plants from similar treatments of the same variety of rose. Under field conditions temperature and moisture of the soil and air are not readily under control to insure optimum conditions for the growth of roses—plants readily injured by less than optimum growing conditions. The control plants, with very low reserve after storage, owing to the number of shoots re-

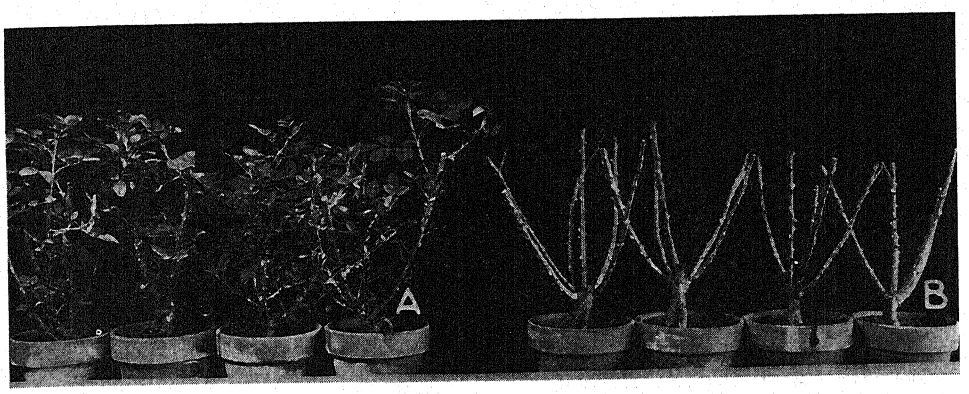


FIG. 4.—Growth of plants (Ami Quinard) after removal to greenhouse, showing continued inhibition of buds by treatment in storage. *A*, control; *B*, treated by spraying with wax emulsion of 0.01 per cent naphthalenemethylacetate. Sprays applied February 26, 1941; plants held in common storage until April 18. Photograph taken after 14 days in greenhouse.

moved before transplanting, grew poorly when field planted, whereas the lots receiving growth substances made excellent growth.

the stock portion of the plant, where there is thicker and more suberized bark than on either the roots or the canes of rose plants.

TABLE 1

EFFECTIVENESS OF COMPOUNDS IN INHIBITING SHOOT GROWTH OF AMI QUINARD ROSE BUSHES HELD IN COMMON STORAGE, AND SUBSEQUENT SHOOT GROWTH ON SAME PLANTS AFTER 21 DAYS IN GREENHOUSE AT 65°–70° F.

CHEMICAL COMPOUND	MEAN NUMBER OF SHOOTS PER PLANT DEVELOPED IN STORAGE (22/6 TO 4/18)*			MEAN NUMBER OF NEW SHOOTS PRODUCED PER PLANT AFTER 21 DAYS IN GREENHOUSE (4/18 TO 5/8)		
	PERCENTAGE CONCENTRATION			PERCENTAGE CONCENTRATION		
	0.05	0.01	0.005	0.05	0.01	0.005
α-naphthalenemethylacetate	0.00	0.00	0.00	27.55	35.60	44.95
α-naphthalenethylacetate	0.00	0.00	0.00	26.90	34.15	49.10
α-naphthaleneacetonitrile	0.00	0.00	11.10	21.45	39.10	47.85
α-naphthaleneacetic acid	0.00	0.00	12.95	31.50	48.75	49.80
α-naphthalene acetamide	0.00	4.25	30.40	46.40	49.70	45.85
β-indoleacetic acid	0.00	5.65	38.20	42.75	46.50	52.25
β-indolebutyric acid	0.00	12.60	40.45	45.40	52.30	50.65
Potassium naphthaleneacetate	0.00	15.65	41.70	42.60	45.70	36.40
Sodium naphthaleneacetate	0.00	19.85	48.35	32.60	38.75	31.55
β-naphthoxyacetic acid	19.90	35.75	48.50	40.65	35.45	38.10
β-indolepropionic acid	25.75	36.10	70.65	51.60	56.80	35.45
α-naphthalenemethylthiocyanate	26.40	38.70	45.85	42.60	47.85	40.30
α-naphthalenemethylisothiocyanate	29.75	39.35	41.50	42.60	47.45	39.60
Tetralin-6-acetamide	35.60	54.15	60.40	42.70	36.85	31.70
Phenylacetic acid	41.60	49.90	68.45	37.90	40.05	24.60
α-naphthalenethio acetamide	42.30	49.95	48.10	32.60	38.75	31.55
α-naphthalenepropionic acid	43.10	49.80	47.65	38.70	39.45	42.50
Control (emulsion spray only)		48.10			40.60	
Control (not sprayed)		40.55			47.25	

* Mean of 20 plants. Differences necessary for significance at: 5% level..... 6.8 | 5% level.....21.5
1% level.....11.2 | 1% level.....29.7

EFFECT OF GROWTH-SUBSTANCE WAX-EMULSION SPRAYS ON BUD INHIBITION WHEN APPLIED TO TOPS ONLY, TO STOCKS ONLY, AND TO ROOTS ONLY

Experiments were undertaken to determine whether application of the wax-emulsion growth sprays to the roots or to the stock portion of plants would be effective in inhibiting shoot formation. There would appear to be less likelihood of injury, especially with applications to

SNOW (13) and WENT (16) have reported that bud inhibition was increased with increased distance from the source of the inhibiting substance. SNOW used the effect on the apical bud itself as a measure of inhibition of lateral buds. WENT used indoleacetic acid-lanolin paste in his experiments on etiolated pea stems. If their findings should hold for the rose bushes, the implication is obvious that such treatments would be most

effective if made either to the roots or to the stock portion of the plant rather than to the canes. The work of STUART (14) and others with cuttings has also demonstrated that the inhibiting effect of growth substance will move upward in plants, since the cuttings were treated at the base and the terminal buds above were inhibited.

To test the inhibiting effect on buds when the substances were applied to parts other than stems, four lots of twenty plants each, similar to those used in the previous wax-emulsion spray experiment, were selected and handled in a similar manner so that direct comparison could be made. One lot of twenty plants received 0.01 per cent naphthaleneacetic acid in wax emulsion sprayed on the stem portion of the stocks only. The roots and tops were protected from the spray by covering them with heavy waxed paper sealed tightly with adhesive tape. Another lot received the same spray applications to the roots only, by similarly protecting the stems of the stocks and canes. A third lot received the spray application to the tops only, the roots and stocks having been covered with sand-peat mixture overlaid with sealed wax paper. In addition, an unsprayed control lot was included.

In setting up the experiment, the surface area of top to roots was adjusted by pruning, so that the amount of spray which adhered to these two parts was fairly comparable. No such adjustment could be made with the surface area of the stock portion of the plants, except as could be done by selecting those with long stem stocks—6–8 inches in length. The plants receiving spray application to this portion only necessarily received somewhat less growth substance.

The sprays were applied on February 26, 1941, and the plants were placed in common storage immediately after treatment. Data obtained on mean number of shoots developed on plants in the different treatments by April 18 indicated that treatment was most effective when applied directly to the vegetative buds, as shown by the following summary of the results: spray applied to tops only, 0.00 shoots; to stocks only, 38.65 shoots; to roots only, 42.68 shoots; control (not sprayed), 40.55 shoots.

Root growth was apparently inhibited in plants that received spray to the roots only, for at the end of the storage period the roots of these plants were callused at the cut surfaces but had produced no new root growth; all other lots had new roots showing on each plant. BONNER and KOEPFLI (2) have also noted inhibition of roots from auxin application.

USE OF VAPORS OF GROWTH-REGULATING COMPOUNDS

Other experiments (17, 5) had indicated that under greenhouse temperature conditions sufficient volatilization of naphthalenemethylacetate will take place to cause growth curvature responses on herbaceous plants and bud inhibition of potato tubers without the application of additional heat to cause rapid vaporization of this compound. The rate of volatilization under the relatively low common-storage temperatures would be slow in comparison with normally high greenhouse temperatures.

Forty rose plants were therefore used to determine whether slow continuous volatilization of growth substance would cause bud inhibition with plants in common storage. The experiment was started March 27, 1940. Two lots of twenty plants each were placed in separate 20-gallon refuse cans having tight-fitting lids. Thirty-five mg. of naphthalenemethylacetate absorbed on a piece of paper

toweling was suspended 1 foot above the plants in a can. Plants in another can served as controls. The cans were sealed and placed side by side in common storage. When opened on April 15 (19 days after treatment), the controls had produced an average of 9.3 shoots per plant, varying in length from 1 to 12 inches, whereas the plants exposed to the vapors were completely dormant. Ten plants were removed from each can at this time, with the etiolated shoots removed from the controls, and placed in the greenhouse for observation, while the remaining ten plants were left in common storage after resealing the cans but without adding more growth substance to the toweling. After 16 days (May 1) in the greenhouse both control and treated lots had made vigorous growth and were very similar in appearance. No evidence of plant injury attributable to the growth substance was found.

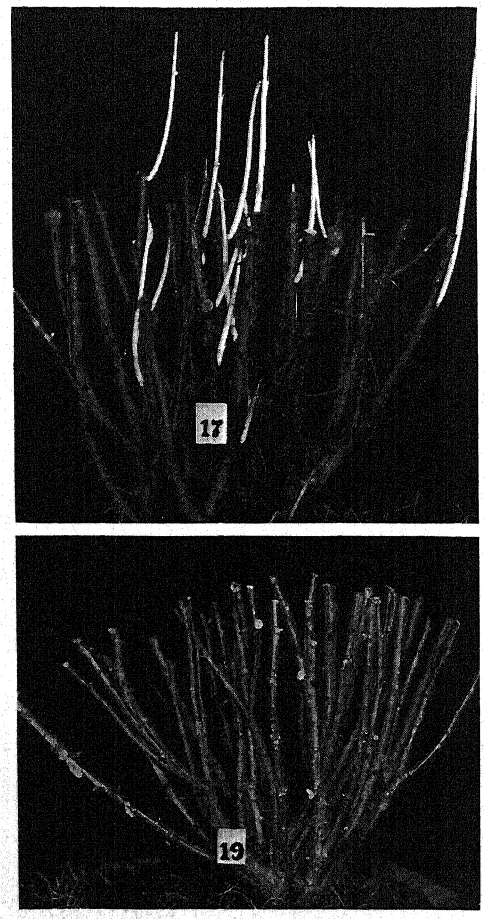


FIG. 5.—Effect of treatment on mold growth: 17, control plants; 19, plants treated with regulating substance. Both lots held in common storage. Treatment began March 27, 1940, when both lots were dormant; they were photographed May 1. Controls show much more shoot and mold growth than treated plants.

The remaining plants that had been left in common storage were removed from the cans on May 1. Differences in response of typical control and treated plants are shown in figure 5. It is apparent that the treated plants were just starting active growth at this time, whereas the controls had produced long etiolated shoots. Mold growth also was apparently inhibited by the vapors. This suppression of mold growth may possibly have been due to a better callusing of the cut surfaces shown in the treated lot or to a direct inhibiting action by the vapor treatment.

EFFECT OF CONCENTRATION OF VOLATILIZED GROWTH SUBSTANCES ON DORMANCY, CANE INJURY, AND GROWTH OF MOLDS

Preliminary treatments with the vapors of several substances in 1939–1940 indicated that three compounds, naphthalenemethylacetate, naphthalenethylacetate, and naphthaleneacetonitrile, were outstanding and about equal in effectiveness in inhibiting the growth of vegetative buds on rose bushes in common storage. Further to test these three compounds for better evaluation, they were again used in 1940–1941 in a wider range of concentrations and with twenty

plants in each treatment. The vapor of each compound was applied at the rate of 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0, and 3.0 gm. per 1000 cubic feet for 16 hours at 70° F. The plants were treated on March 12, 1941, and placed in common storage. The compounds and treatments used are given in table 2, together with data on shoot production and cane loss found when the plants were examined on May 1.

pounds caused an increase in number of shoots produced per plant in comparison with the controls. From the standpoint of bud inhibition, therefore, these results suggest that of the three compounds applied as vapors the two esters appear the more effective.

Injury from a relatively high concentration of growth substance was noted early in the experiments; it may result in severe cane loss (table 2) while the

TABLE 2

COMPARISON OF VOLATILE GROWTH SUBSTANCES BASED ON SHOOT GROWTH AND CANE LOSS WITH AMI QUINARD ROSE BUSHES HELD IN COMMON STORAGE. TREATED MARCH 12, 1941; DATA OBTAINED AT END OF STORAGE PERIOD (MAY 1)

Concentration of growth substance in grams per 1000 cubic feet (16 hours at 70° F., as gas)	Naphthalenemethylacetate		Naphthalenethylacetate		Naphthaleneacetonitrile	
	Mean number shoots per plant: S.E.	Mean percentage dead canes per plant: S.E.*	Mean number shoots per plant: S.E.	Mean percentage dead canes per plant: S.E.	Mean number shoots per plant: S.E.	Mean percentage dead canes per plant: S.E.
0.100....	90.7±17.87	56.70±11.20	86.4±18.14	65.40± 7.65	74.6± 8.42	49.90±14.81
0.200....	25.6± 5.86	38.80± 8.41	32.5± 9.30	29.90± 6.90	68.5±14.35	46.65±12.45
0.300....	2.0± 0.41	2.75± 0.53	10.3± 2.65	8.25± 1.25	21.8± 7.02	13.36± 1.41
0.400....	0.0± 0.00	11.90± 3.03	0.0± 0.00	15.31± 2.32	3.4± 0.45	4.45± 0.84
0.500....	0.0± 0.00	29.15± 4.97	0.0± 0.00	32.65±16.41	0.0± 0.00	26.35±11.50
1.000....	0.0± 0.00	48.42±12.81	0.0± 0.00	54.40±18.71	0.0± 0.00	60.70±14.40
2.000....	0.0± 0.00	65.75± 2.90	0.0± 0.00	76.74± 4.90	0.0± 0.00	82.50± 2.13
3.000....	0.0± 0.00	90.50± 0.75	0.0± 0.00	84.90± 2.35	0.0± 0.00	98.90± 1.56
Control.	65.8± 7.83	45.8 ± 8.02	.....	.....	.....	.....

*Twenty plants per treatment. Percentage dead canes calculated from length of dead to total cane length per plant.

Comparison of shoot production on the plants on this date (table 2) shows that both esters were more effective than the nitrile, the esters having caused complete bud inhibition when applied at the rate of 0.4 gm. per 1000 cubic feet, while with the nitrile the rate necessary to cause complete inhibition was 0.5 gm. Further, the methyl ester was slightly superior to the ethyl ester (comparing both at the 0.3-gm. concentration). At the lowest concentration (0.1 gm. per 1000 cubic feet), however, all three com-

plants are in common storage. The injury was noticeably different in the control lots as compared with the treated lots. In the former the 45.8 per cent injury (table 2) was largely due to cane destruction by disease organisms, starting in the tender etiolated shoots and progressing into the canes. Material injured by substances applied in strong gaseous concentrations was first evidenced by the appearance of small black sunken areas in the internodal regions at and near the ends of the canes. With the

three compounds applied as gases at high concentrations of 1, 2, and 3 gm. per 1000 cubic feet, injury extended over the entire length of the canes, usually resulting in death (fig. 6*B*).

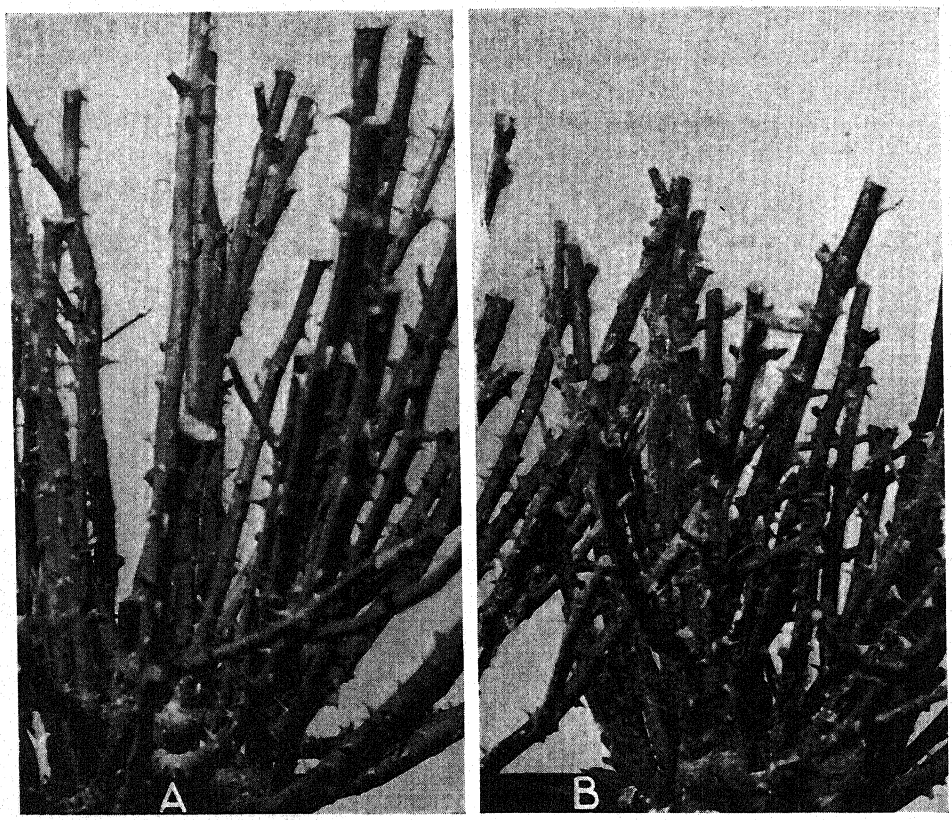


FIG. 6.—Appearance of injury due to highly concentrated volatilized growth substance: *A*, dormant and uninjured Ami Quinard rose stems with slightly callused ends after 60 days in common storage, resulting from low concentration of 0.3 gm. per 1000 cubic feet naphthalenemethylacetate. *B*, almost complete killing of canes as result of application of same compound at rate of 1 gm. per 1000 cubic feet, showing characteristic black pitting type of injury.

The reduction in amount of cane loss found on plants treated with 0.3 gm. naphthalenethylacetate per 1000 cubic feet would suggest that this compound had some direct fungicidal action (fig. 6*A*). To determine this, media were prepared by cutting the canes into $\frac{1}{4}$-inch lengths, boiling them in water in 200-cc. flasks for 1 hour, and autoclaving to sterility. These media were then transferred to large 9-inch sterile culture dishes, so that a single layer of the cane pieces covered the bottom of each dish. The dishes were then inoculated with a pure culture of *Botrytis* sp. (of the cinerea type),[2] a mold organism obtained from badly infected rose bushes.

After standing overnight, the mold had developed and covered an area about 1 inch in diameter. Ten dishes for each

[2] Dr. J. S. COOLEY kindly prepared the pure culture of this organism and was freely consulted on the pathological phases of the problem.

treatment were then selected and treated with naphthalenethylacetate at the rate of 0.3, 0.5, and 1 gm. per 1000 cubic feet, respectively, for 17 hours at 70° F. Subsequent to treatment, the dishes, together with ten inoculated controls, were moved into the common storage room, which was maintained at 38°–42° F.

The time, in days, required for mold completely to cover the media in the bottom of the dishes was used as an index of the effectiveness of treatment. The following results were obtained: The control dishes developed mold growth in 2–4 days, with a mean of 2.5 days; 0.3-gm. concentration of growth substance ranged 12–15 days, with a mean of 12.4 days; 1-gm. concentration ranged 15–21 days, with a mean of 17.7 days. These results indicate that the treatments had pronounced delaying action on mold growth, which is further shown in figure 7. Complete mold destruction, however, was not accomplished in any of the treated dishes. It is possible that the delay in mold growth, amounting to 12 days in the 0.5-gm. treatment and 17.7 days in the 1-gm. treatment, may prove helpful in suppressing mold on plants in common storage. The data in table 2, however, show that application of both concentrations of naphthalenemethylacetate to intact rose bushes resulted in moderate to severe cane injury.

Plants treated with the relatively high concentration of 1.0 gm. naphthalenemethylacetate also showed heavy callus formation in addition to injury. Some callus formation would appear desirable, since it tended to seal the cut ends of the canes and prevent entrance of mold organisms. Too much, however, would deplete stored food reserves similar to depletion caused by shoot growth on the untreated controls.

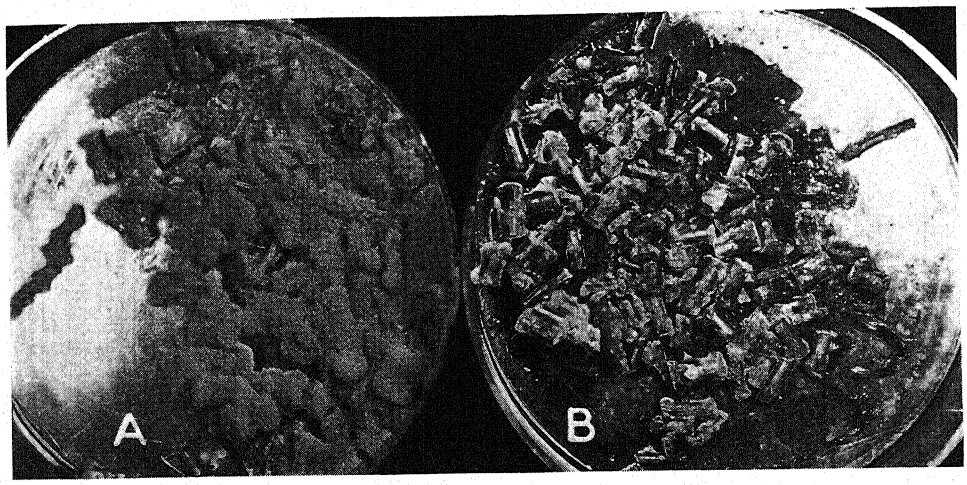


FIG. 7.—Suppression of mold growth on rose cane media by growth substance: *A*, control; *B*, treated with 1.0 gm. naphthalenemethylacetate per 1000 cubic feet. Both lots held in common storage after treatment. Photograph taken after 12 days.

EFFECT OF TEMPERATURE, LENGTH OF TREATMENT INTERVAL, AND CONCENTRATION OF GROWTH SUBSTANCE ON DORMANCY

The temperature within common storage houses fluctuates widely during the storage season. It may extend from about 32° to 50° F. in the same season.

When it is possible to maintain common storage at temperatures of 32°–38° F., the nurseryman usually encounters little difficulty in holding rose bushes in a dormant condition during the normal storage season. Beginning about April 1, however, and in some years somewhat earlier, the temperature of common storage houses rises to 40° F. and above. Rose bushes will show some vegetative activity after prolonged storage, even at 32° F.; at 40° F. and above, shoot production is usually rapid.

In view of the published information relative to the effects of temperature during treatment with growth substance, it would seem necessary—in order to prolong dormancy of rose bushes—either to remove the bushes from storage to a warm room or to raise the temperature of the storage itself during treatment. The former would involve the use of considerable extra labor, since many of the houses normally store up to several million bushes, while the latter method would not be desirable because of the length of time necessary to bring the storage house back to favorable temperature. An experiment with a gaseous method of application was set up to determine to what extent lengthening of the treatment interval or increase in concentration of growth substance might tend to increase the effects under low temperature.

The temperatures selected for treatment were 32° and 40° F., to represent conditions encountered under common storage. Similar sets of plants were treated at 70° F., to represent treatment at normal room temperature. In the experimental set-up, thirty-six lots of bushes, composed of twenty plants per lot, were used. The temperatures, treatment interval, and concentrations of growth substances are shown in table 3, as well as the mean number of shoots produced on plants in the different lots by May 5, 1941. By this date the mean number of shoots per plant on the control lots was: 32° F., 67.0 ± 8.24; 40° F., 62.4 ± 6.90; 70° F., 65.8 ± 7.83.

TABLE 3

EFFECT OF CONCENTRATION OF GROWTH SUBSTANCE, TEMPERATURE, AND LENGTH OF TREATMENT INTERVAL ON NUMBER OF SHOOTS DEVELOPING ON AMI QUINARD ROSE BUSHES DURING COMMON STORAGE. TREATED MARCH 12–17, 1941. MEAN* NUMBER DEVELOPED BY MAY 5

TEMPERATURE DURING TREATMENT	LENGTH OF TREATMENT (HOURS)	MEAN NUMBER OF SHOOTS PER PLANT			
		0.10 GRAM†	0.30 GRAM	0.50 GRAM	1.0 GRAM
32° F.	1	115.25	98.15	90.42	22.65
	4	98.50	40.75	26.47	6.20
	16	79.75	10.30	3.50	3.65
40° F.	1	75.00	28.46	16.00	15.12
	4	80.94	11.25	4.50	0.00
	16	83.60	0.00	1.50	0.00‡
70° F.	1	75.35	12.64	5.24	0.00‡
	4	79.00	7.92	6.95	0.00‡
	16	75.26	3.38	1.25	0.00‡

* Twenty plants per treatment.

† Concentration of naphthalenemethylacetate gas per 1000 cubic feet.

‡ Severe plant injury.

Difference necessary for significance at 5% level, 7.43; at 1% level, 11.93.

The plants showed much variability, as indicated from the fact that at the 5 per cent level differences of 7.4 shoots were required for significance, while at the 1 per cent level this was increased to 11.9 shoots for highly significant differences.

The data in table 3 indicate that treatment will effectively control shoot growth on rose bushes in storage, even though such application is made at 32° F., as both 0.50-gm. and 1.00-gm. concentrations per 1000 cubic feet were effective when applied over a 16-hour pe-

riod. The higher (1.00 gm.) concentration would not be considered safe to use, however, since considerable cane injury took place in several lots receiving this amount of the same substance (fig. 6*B*). Thus treating at 32° F., the 0.3–0.5-gm. concentration with 16-hour treatment interval would appear preferable, since it was equally effective and not so likely to induce injury.

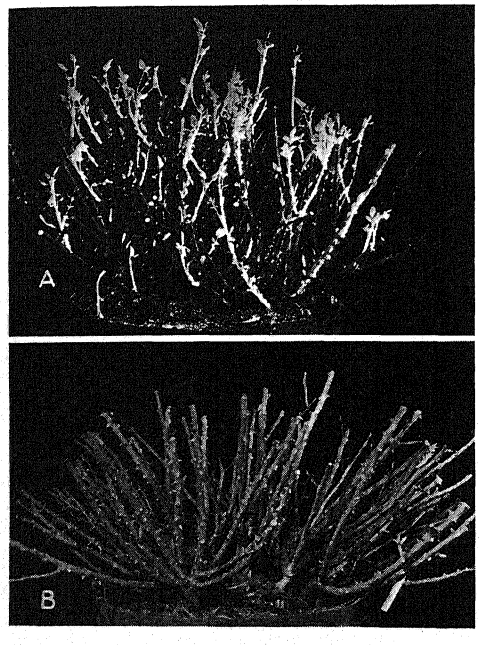


FIG. 8.—Effect of temperature in storage house on response to treatment: *A*, control plants (Ami Quinard); *B*, similar plants treated with 0.3 gm. naphthalenemethylacetate for 16 hours at 40° F. Treated March 17, 1941, and both lots held in common storage. Photographs taken May 1.

Bushes treated at 40° F. were kept completely dormant by the 0.3-gm. concentration applied over a 16-hour period (fig. 8). Similar plants treated for 4 hours with 0.5 gm. of the same substance were not significantly different in shoot growth at the 1 per cent level.

No significant benefit in shoot inhibition was found from extending the treatment interval beyond 1 hour when growth substance was applied at 70° F., except possibly in comparing 1 hour with 16 hours at the 0.3-gm. concentration (table 3).

Applications of 0.10 gm. of naphthalenemethylacetate at each of the three temperatures and for each of the three intervals of treatment not only did not inhibit shoot production but appeared to cause stimulation (fig. 9), as comparable control plants included in another experiment produced a mean of 65.8 shoots per plant. A similar effect has been noted

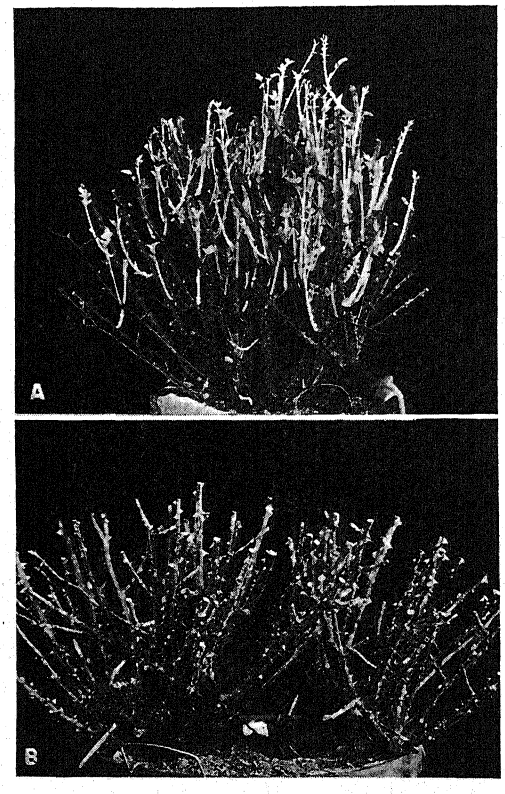


FIG. 9.—Stimulating effect on shoot production of low concentration and injurious effect of high concentration of growth substance: *A*, Ami Quinard plants treated with volatilized naphthalenemethylacetate, 0.10 gm. per 1000 cubic feet for 1 hour at 32° F.; *B*, similar plants severely damaged by 1.0 gm. per 1000 cubic feet for 16 hours at 40° F.

by BENNETT and SKOOG (1) and by VEGIS (15). The most pronounced stimulation occurred in plants treated with 0.10 gm. of growth substance for 1 hour at 32 F. In this lot almost every bud appeared to break and produce shoots, so that by the end of the storage period a mean of 115 shoots per plant had developed (fig. 9).

EFFECT OF APPLYING GROWTH-REGULATING SUBSTANCE IN PARTIAL VACUUM

A partial vacuum was used to determine whether, by causing better gaseous penetration of the growth substance between the bud scales of dormant buds, the effectiveness of a given concentration might be increased. This would permit the more effective use of a lower concentration of growth substance, with the added advantage of little or no plant injury.

TABLE 4

MEAN NUMBER OF SHOOTS ON TWENTY ROSE BUSHES HELD IN COMMON STORAGE 60 DAYS, FOLLOWING TREATMENT WITH GROWTH SUBSTANCE VOLATILIZED IN PARTIAL VACUUM

CONCENTRATION OF NAPHTHALENETHYLACETATE (GM. PER 1000 CUBIC FEET)	NORMAL ATMOSPHERE PRESSURE 17 HOURS		PARTIAL VACUUM 17 HOURS	
	MEAN	S.E.M.	MEAN	S.E.M.
0.100.....	90.7	±17.87	82.4	±19.42
0.200.....	25.6	± 5.86	20.3	± 6.98

Two lots of bushes of twenty plants each were treated with naphthalenemethylacetate gas in a partial vacuum of 0.7 atmosphere at the rates of 0.1 and 0.2 gm. per 1000 cubic feet, respectively, for 17 hours at 70° F. The lower concentration was applied on March 11, the higher on March 12.

As shown in table 4, treatment in partial vacuum did not increase significantly the effectiveness of low concentration of substance. There was a slight reduction in actual number of shoots in both lots treated in partial vacuum, which may indicate a tendency for slight beneficial effect. In connection with other experiments, it was noted that the gas appeared to condense on surfaces nearest the gas source. Considering the very minute quantities used, it is possible that this may have occurred and offset any advantage obtained from reducing the atmospheric pressure.

EFFECT OF GROWTH-REGULATING SUBSTANCE ON RELATIVE RATE OF STARCH DISAPPEARANCE IN PLANTS DURING COMMON AND COLD (32° F.) STORAGE

The growth of plants after storage is often materially influenced by the amount of stored reserves remaining in them at the end of the storage period. An experiment was conducted to determine whether growth-regulating substance would influence the rate of starch loss during storage.

Plants that had received a gaseous application of naphthalenemethylacetate at the rate of 0.5 gm. per 1000 cubic feet for 16 hours at 70° F. on March 10, 1941, were placed in both common and cold (32° F.) storage, together with comparable untreated controls. Periodically at 2-week intervals thereafter (until May 5) twenty plants were removed from each lot and estimations made of the relative starch content of the individual plants by the IKI method previously described.

As shown in figure 10, rapid disappearance of starch had taken place from the stocks of the common storage control plants 14 days after treatments were applied. Apparently the experiment was started very near the critical period for starch disappearance. A proportionally greater amount of starch was found

stored in the wood of the stock throughout the entire storage period than in either bark or pith tissues. The small amount present in the bark was first to disappear, and growth substance apparently had slight or no effect on its rate of loss.

The rate of disappearance of starch from the wood with plants held in common storage was markedly reduced by growth-substance treatment, which was similar to the reduction in starch loss resulting from storage at 32° F. No visible sign of bud breaking or root growth was evident on even the common-storage controls until after the fourteenth day of treatment. It would appear likely that growth substance had affected those processes which bring about starch hydrolysis or which utilize starch in respiration. The large quantity of shoots formed on the common storage plants later would account for the ultimate loss of much of the stored reserves, whether originally present as starch or in some other form.

Cold storage at 32° F. is considered near the ideal temperature for rose bushes. Treatment with growth substances showed no significant effect on the starch present in plants held at this temperature, until near the end of the storage period. At the last two sampling dates (fig. 10), starch was significantly higher in the wood and pith of treated plants than of comparable controls held at 32° F. At the last sampling date, however, the cold-storage controls were showing visible vegetative signs of growth, even at this low temperature. The treated lots were completely dormant in both common and cold storage until after field planting.

Application of growth substances to rose bushes in order to conserve their starch content would appear to be of questionable benefit if plants are to be held in 32° F. storage. Subsequent behavior of similar plants that were field planted, however, would seem to justify such treatment, since the treated plants became established in the soil more quickly and made better growth. Data to support this conclusion are presented with the field-planting responses obtained.

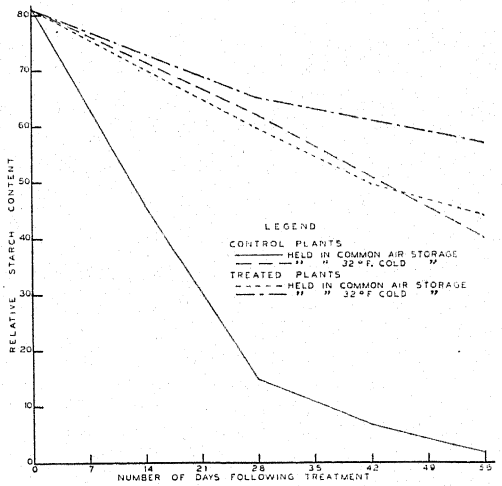


FIG. 10.—Rate of starch disappearance in woody portion of stock of Ami Quinard rose bushes held in common and in cold storage following growth-substance treatment.

Probably the most significant effect, at least from a practical standpoint, is the close similarity of the starch content of plants treated with growth substance and held in common storage with control plants held in 32° F. storage (fig. 10). This effect, however, was true only for starch stored in the wood portion of the stock, and did not hold to the same degree for starch found in the bark and pith tissues.

RESPONSES OF FIELD-PLANTED BUSHES FOLLOWING GROWTH-SUBSTANCE APPLICATION AND SUBSEQUENT COMMON AND COLD STORAGE

Included in each lot of plants used for the relative starch tests previously de-

scribed was an additional lot treated and stored in identically the same way. After storage these plants were used in a field planting to determine whether any effects of the different storage treatments would be reflected in root and shoot growth as well as blossom production.

The plants were uniformly root pruned to six primary roots per plant and planted in the field on May 5. The twenty

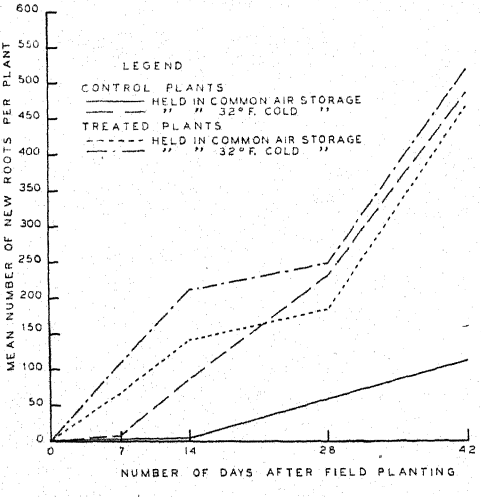


FIG. 11.—Relative rate of new root production on field-planted Ami Quinard rose bushes held in common and in cold storage with and without growth-substance treatment. Field planted May 5, 1941, 56 days after storage treatments were started.

bushes of each of the four lots (treated and controls from common and cold storage) were set at uniform depth, with the roots of each plant oriented in the same relative positions. This systematic spacing of the roots greatly aided in relocating them to obtain counts on new root production.

The very poor production of new roots early in the growing season on the control plants held in common storage (fig. 11) may possibly account for the loss of seven of the twenty plants included in this lot. Evidently the stored food reserves had been depleted to such a low level, as indicated by the starch test made just prior to field planting, that insufficient quantity was available to supply demands for both new root and new shoot growth. On these plants shoots developed before new roots were initiated, as shown by comparing common storage controls in figures 11 and 12. It is possible that roots were delayed in formation until the newly formed leaves could supply the necessary materials for root growth.

Treatment of plants in both common and cold storage was followed by significantly more root development during the first week in the field than on similar untreated plants (fig. 11). This effect was greatest with plants held in cold storage. Such plants had developed 110 roots per plant 7 days after planting, as compared with 68 per plant following treatment in common storage.

As shown previously (fig. 10), the control plants held at 32° F. had approximately the same relative starch content as the treated common-storage plants (39.5 and 43.7, respectively) at the time of field planting. Theoretically they should be able to produce about the same amount of growth from their stored reserves. At the end of the first week in the field the cold-storage controls had developed an average of six roots per plant, whereas the treated lots from common storage averaged 68 roots per plant (fig. 11). On the other hand, the buds of the cold-storage controls developed rapidly after field planting and at the end of the first week had produced an average of 48.5 mm. of new shoots as compared with only 9.2 mm. in the treated lots from common storage. Apparently stored reserves were being used up in top growth in the one case, while in the other new roots were becoming established.

One explanation of the stimulative ac-

tion of growth substances on root production would appear to be a direct effect on the roots themselves, as has been reported for young pecan trees (11). Data obtained in the present experiments would indicate that the effect may be an indirect one. It would seem that the main effect of growth-substance treatment under the conditions of this experiment resulted from inhibition of shoot growth for a short interval after field planting rather than from direct stimulative action on the roots themselves.

The common-storage control plants also made much poorer top growth than those of any other treatment throughout the growing season. Controls from cold storage made an initial flush of growth that was greater than in any other treatment. However, treated lots from both common and cold storage caught up with them by the end of 21 days in the field, and at the end of 42 days they had produced almost twice as much top growth (fig. 12). The common- and cold-storage treated lots were very similar in amount of growth at this time, with 1181 mm. per plant in the former and 1.19 cm. in the latter lots. Controls held in cold storage had grown but 6 cm. by this date.

It would appear that application of growth substance to dormant rose bushes may be followed by better growth after planting in the field. The results obtained indicate that the response is not caused by a direct beneficial action on growing shoots.

In table 5 weekly records of blossom production taken from June 18 until October 24 are summarized by months on the same plants as used in the root and shoot growth studies.

As a result of slight root and shoot growth, flower production on the common-storage control plants was low, and the flowers had very short stems. Since the cold-storage control plants started growth earlier than those of other treatments, it would seem likely that these plants would blossom sooner. Such was not the case. Records during the month of June show that treated lots, both in common and cold storage, had produced

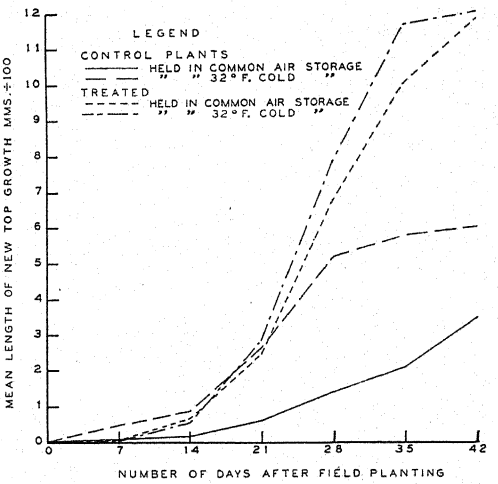


FIG. 12.—Relative rate of new top growth on field-planted Ami Quinard rose bushes held in common and in cold storage with and without growth-substance treatment. Field planted May 5, 1941, 56 days after storage treatments were started.

more blossoms early in the season. The flowers also had longer stems, particularly those from plants treated and held in cold storage.

Both control lots, common and cold storage, ceased flower production earlier and went into fall dormancy sooner than either of the treated lots. This effect is shown to some extent by the lessened flower production of the controls during October (table 5), as well as by the striking differences in amount of foliage shown in figure 13. This response may be due to a difference in vigor of plants at this time, comparing control and treated lots, rather than to any direct

effect of growth substance in this connection.

It is obvious that cold storage alone was not sufficient to obtain optimum flower production. Plants that had received a combination of cold storage with growth-substance treatment produced twice as many flowers per plant during the growing season as did those receiving cold storage alone; the former produced thirty-one flowers per plant, the latter fifteen. Intermediate were the plants held in common storage with growth-substance treatment. These averaged twenty-one flowers per plant, many of which were small with short stems.

TABLE 5

FLOWER PRODUCTION OF FIELD-PLANTED AMI QUINARD ROSE BUSHES FOLLOWING TREATMENT WITH GROWTH SUBSTANCE IN COMMON AND IN COLD STORAGE

TREATMENT*	NO. OF PLANTS†	MEAN S.E.M.					
		JUNE 18 TO 30	JULY 1 TO 31	AUGUST 1 TO 31	SEPTEMBER 1 TO 30	OCTOBER 1 TO 24	TOTAL
Control plants; common storage	13	0.23±0.12	2.00±1.01	2.00±0.00	4.30±0.63	.77±0.15	9.50±2.71
Growth-substance treated; common storage...	20	3.90±1.01	4.70±1.42	1.10±0.43	8.80±2.90	3.25±1.35	21.75±6.45
Control plants 32° F.; cold storage.....	20	1.75±0.46	5.50±1.78	.50±0.13	6.05±3.01	1.60±0.52	15.40±4.30
Growth-substance treated 32° F.; cold storage....	20	4.25±1.98	8.30±2.41	1.50±0.54	13.40±6.25	3.55±1.21	31.00±9.45

* Growth substance = 0.5 gm. naphthalenemethylacetate per 1000 cubic feet for 16 hours at 70° F.

† Number surviving out of 20 planted.

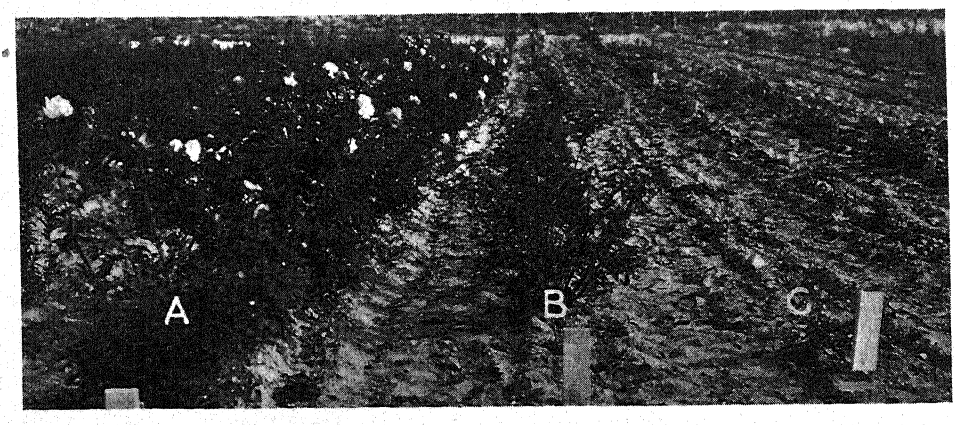


FIG. 13.—Relative size of plants and differential rate of defoliation on October 20, 1941, of Ami Quinard rose bushes field planted May 5, which had received growth-substance treatment: *A*, foreground, common storage plus growth substance; background, cold storage plus growth substance. *B*, cold-storage controls. *C*, common-storage controls. The two rows to right of *C* are composed of plants treated with strong concentrations of 1, 2, and 3 gm. growth substance per 1000 cubic feet.

Under the conditions of this experiment, treatment at the beginning of the common- and cold-storage period has improved the flower-producing capacity of rose bushes. Although treatment was very effective on plants held in cold storage, even greater relative difference was found with plants held in common storage, compared with the respective control plants.

Effect of growth-regulating substance on bud inhibition of fifteen varieties of roses

Although the experiments were mainly concerned with the Ami Quinard rose, a hybrid tea variety that tends to break dormancy early in storage season, tests with growth-regulating substance were also conducted with ten other hybrid tea varieties (Editor McFarland, Edith Nellie Perkins, Etoile de Hollande, Duquesa de Penaranda, Girona, Golden Dawn, Margaret McGredy, Radiance, Radio, and Ramon Bach); also three polyantha varieties of rose (Chatillon, Topaz, and Poulsen's Yellow), as well as the variety Guinee, a climbing type.

These experiments will be reported in more detail elsewhere. It may be mentioned, however, that the vegetative buds of all three types were inhibited to some degree by treatments effective with the Ami Quinard variety. Shoot development of the climbing rose appeared to be least difficult to inhibit, and the canes appeared to be least susceptible to injury from strong concentration of the substance. In the case of the hybrid tea varieties some variation was noted among them in the ease of control of shoot development in storage. Further, two of the ten hybrid tea varieties (Duquesa de Penaranda and Golden Dawn) appeared to be more readily injured by treatment than the others. Treatment with a gaseous application of naphthalenemethylacetate at the rate of 0.3 gm. per 1000 cubic feet for 16 hours at 70° F. appeared to be about optimum, however, markedly reducing shoot development without causing noticeable cane injury with all ten varieties. The three varieties of polyantha rose responded alike to treatment. All developed some shoot growth when treated with applications of substance effective in the bud inhibition of both hybrid tea and climbing varieties. The amount of shoot development, however, even with the polyantha varieties, was significantly reduced when treated and held for 2 months in common storage.

Possible polyploidal or formative effects induced by application of strong concentration of growth-regulating substance

Several investigators have reported that growth-regulating substance will induce polyploidy (3, 4, 7) and cause formative effects (18) on plants other than the rose. Consequently, all the rose bushes that had received strong (1 gm. or more per 1000 cubic feet of volatilized compound) dosages of growth substances in any of the storage experiments previously described were field planted, even though many had as much as 90 per cent of their tops severely injured. These plants were set in two rows adjacent to the rose planting used in obtaining the growth-response data already mentioned. As shown in figure 13, many of these plants had died by October 20, 1941. Those that lived throughout the growing season were carefully observed for any abnormalities in plant character or flower structure. The few flowers that were produced were normal and the plants themselves did not appear to be at variance with comparable weak-growing control

plants that had been used in the common-storage experiments.

When the plants were dug in late November, it was noted that many large buds had apparently remained dormant at the base of the larger canes throughout the growing season. These buds were present on the plants when they had been treated with growth substance during their previous storage period. It seemed advisable, therefore, to attempt to force them to develop. After potting the plants in soil on November 24 they were moved into a greenhouse at 32°–45° F. to complete their dormancy. Following this period, they were forced in a warm greenhouse, together with similar control plants.

Figure 14 shows a flower resulting from treatment, considerably different from the varietal form, having 150 petals. Counts on twenty-five flowers from control plants ranged in number from 65 to 80 petals each. Another plant of the same variety also produced a flower with apparently many more petals than those on the control or treated plants.

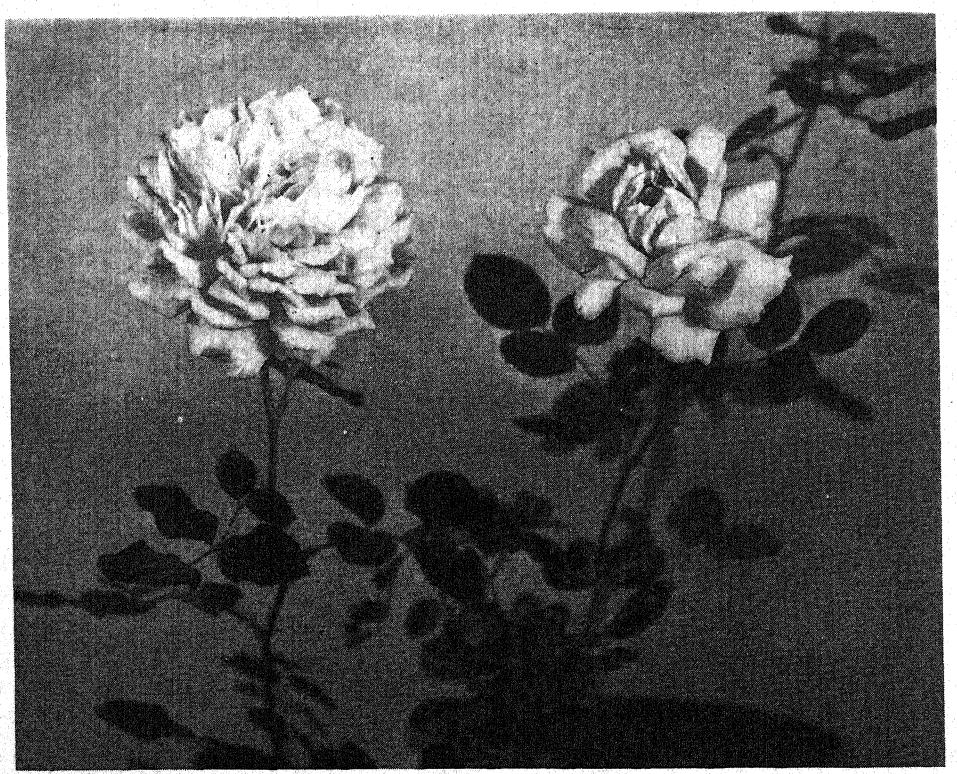


FIG. 14.—Left, flower of Ramon Bach variety of rose, having 150 petals, following treatment with 1 gm. gaseous naphthalenemethylacetate per 1000 cubic feet. Right, flower from control plant of same variety, with 70 petals.

The results obtained in the present experiments indicate that changes in the varietal characteristics of rose plants following treatment with growth substance to inhibit shoot formation in storage are

not likely to occur unless strong concentrations of the active compounds are used.

Discussion

The evidence obtained in these experiments on roses is in agreement with WENT's (16) finding that bud inhibition is greatest when a continuous supply of growth substance is available. The increased effectiveness of such substances when applied in wax emulsion as compared with comparable concentration applied in aqueous solutions was apparently due to the growth substance being condensed and held on the plant surface over a longer period of time. If penetration by strong concentration in a short time were a primary factor in bud inhibition, it would seem that the aqueous solutions, as well as sprays containing a penetrating agent (such as ethyl alcohol), have an advantage, since the plants would be able to take up growth substance from such sprays quicker than from the wax-emulsion sprays. This was not the case, nor did increasing the penetration of the volatile growth substance (naphthalene-methylacetate) by applying vapor treatment to plants in a partial vacuum show increased effectiveness on bud inhibition over treatment under atmospheric pressure with the same rate of application.

ZIMMERMAN and HITCHCOCK (17) have suggested that the increased effectiveness of vapor over solution treatments with the same growth substance, as has been indicated in the present experiments, was due primarily to increased penetration by the vapors. They considered that vapor penetration took place in a manner similar to that of ethylene gas, used in some of their earlier experiments. This conclusion appears logical, especially since they obtained relatively more rapid and pronounced effects from the vapors under the greenhouse conditions and with the vegetative plants they used. In the case of the dormant rose plants of the present experiments, held in common storage at 32°–42° F., however, the increased effectiveness of vapor treatments appeared to arise largely from the fact that the vapor condensed in tiny droplets on the plant surfaces. Slow volatilization from these droplets under the low temperature conditions employed appeared to be a primary factor in the bud-inhibiting effects obtained.

Injury usually followed when strong concentrations of growth substances were applied to dormant rose plants. The injury did not appear to be confined to the buds but showed in damage to the bark and cambium of the internodal regions on the canes. Evidence of this was found upon removal of the injured plants to the greenhouse, where many of the buds on such plants started growth but subsequently died because of a girdling effect of the injured tissues above and below the vegetative buds. This is in contrast with the direct bud injury obtained with peach and cherry material included in experiments not reported here, as well as that obtained by MITCHELL and CULLINAN (8) with peach and pear. The bud scales on most rose varieties seem to fit tightly together and are waxy when the plants are dormant. Entrance of growth substance as vapors into the plant would therefore seem most likely through the lenticels of the bark rather than directly into the buds. The first noticeable sign of cane injury appeared as small sunken black pits in the bark. These pits seemed to be centered around the lenticel openings. As injury progressed the pits expanded in circumference until they merged, forming a continuous sunken black area.

Indoleacetic acid, reported as effective in bud inhibition on herbaceous plants (16, 12), was relatively ineffective with the woody rose plants. WENT (16), however, has shown that with this compound the concentration necessary for bud inhibition was very close to the concentration which induced injury. It is possible that inhibition without injury might have been obtained on the rose plants if a concentration of indoleacetic acid intermediate between 0.05 and 0.01 per cent had been used.

MITCHELL, KRAUS, and WHITEHEAD (9) and MITCHELL and WHITEHEAD (10) have found that under some conditions application of growth-regulating substances to bean leaves may have a stimulating effect on the rate of starch hydrolysis, while under other conditions the applications have no effect. The present experiments indicate that with dormant rose plants stored at relatively low temperatures application of growth-regulating substances may have a retarding effect on the rate of starch hydrolysis. The greatest retardation in starch loss was obtained with treated (0.5 gm. methyl ester per 1000 cubic feet for 16 hours at 70° F.) plants held in cold storage at 32° F. With plants held in common storage, treatment retarded the loss of starch, so that at the end of the storage period there was no significant difference in relative starch content, as indicated by the IKI test, between these plants and similar untreated ones held continuously in cold storage (32° F.). The untreated plants during common storage produced a greater number of etiolated shoots per plant than the treated, however, so that it would be difficult to estimate how much of the reserve starch supply was utilized in producing shoots and how much was conserved by direct action of the growth substance on starch hydrolysis.

Summary

1. Experiments conducted with rose bushes during common storage in two seasons, 1939–1940 and 1940–1941, indicate that growth-regulating substances will inhibit the vegetative buds so that the plants remain dormant throughout the normal storage season. Approximately 4400 rose bushes were utilized in experiments with seventeen chemical compounds.

2. Three compounds (naphthalenemethylacetate, naphthalenethylacetate, and naphthaleneacetonitrile) effectively inhibited growth of buds for 40–60 days in common storage. Application of these compounds was effective when applied at concentration of 0.01 per cent in 0.25 per cent wax-emulsion sprays applied to plant tops, or as vapors to dormant plants at rate of 0.3 and 0.4 gm. per 1000 cubic feet.

3. The vapor of naphthalenemethylacetate at the rate of 0.3 gm. per 1000 cubic feet was equally effective on bud inhibition when applied at 32° F. for 16 hours, 40° F. for 4 hours, and 70° F. for 1 hour.

4. Concentrations of a naphthalenemethylacetate vapor stronger than 0.5 gm. per 1000 cubic feet caused moderate to severe cane injury, while a lower concentration of 0.1 gm. per 1000 cubic feet increased the number of shoots developing in storage.

5. Applications in general appeared to be most effective when a continuous supply was present at or near the inhibited buds.

6. Growth-regulating substances may have an inhibiting action on mold growth, either through mild direct fungi-

cidal action or by reducing the number of etiolated shoots which establish ready access for mold infection.

7. The food reserves (starch) of treated bushes were conserved while in storage, either by direct inhibition of the treatment on starch hydrolysis or by prevention of shoot growth which utilizes the available reserves. As a result, treated plants after storage produced much greater root and top growth and more flowers of better quality per plant when field planted than the untreated controls.

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LITERATURE CITED

1. BENNETT, J. P., and SKOOG, FOLKE, Preliminary experiments on the relation of growth-promoting substances to the rest period in fruit trees. Plant Physiol. **13**:219–225. 1938.
2. BONNER, JAMES, and KOEPFLI, J. B., The inhibition of root growth by auxins. Amer. Jour. Bot. **26**:557–565. 1939.
3. DERMEN, HAIG, Intranuclear polyploidy in bean induced by naphthalene acetic acid. Jour. Hered. **32**:133–138. 1941.
4. GREENLEAF, W. H., Induction of polyploidy in *Nicotiana*. Jour. Hered. **29**:451–464. 1938.
5. GUTHRIE, J. D., Inhibition of the growth of buds of potato tubers with the vapor of the methyl ester of naphthalene acetic acid. Contrib. Boyce Thompson Inst. **10**:325–328. 1939.
6. JONES, S. E., and RICHEY, H. W., The use of wax emulsion in reducing desiccation of transplanted tomato plants and apples in storage. Proc. Amer. Soc. Hort. Sci. **36**:751–753. 1939.
7. LEVAN, ALBERT, Cytological phenomena connected with the root swelling caused by growth substances. Hereditas **25**:80–96. 1939.
8. MITCHELL, J. W., and CULLINAN, F. P., Effects of growth-regulating chemicals on the opening of vegetative and floral buds of peach and pear. Plant Physiol. **17**:16–26. 1942.
9. MITCHELL, J. W., KRAUS, E. J., and WHITEHEAD, MURIEL R., Starch hydrolysis in bean leaves following spraying with alpha naphthaleneacetic acid emulsion. BOT. GAZ. **102**:97–104. 1940.
10. MITCHELL, J. W., and WHITEHEAD, MURIEL R., Starch hydrolysis in bean leaves as affected by application of growth-regulating substances. BOT. GAZ. **102**:393–399. 1940.
11. ROMBERG, L. D., and SMITH, C. L., Effects of indole-3-butyric acid in the rooting of transplanted pecan trees. Proc. Amer. Soc. Hort. Sci. **36**:161–170. 1939.
12. SKOOG, F., and THIMANN, K. V., Further experiments on the inhibition of the development of lateral buds by growth hormone. Proc. Nat. Acad. Sci. Washington **20**:480–485. 1934.
13. SNOW, R., Experiments on growth and inhibition. I. The increase of inhibition with distance. Proc. Roy. Soc. London B **108**:209–223. 1931.
14. STUART, N. W., Nitrogen and carbohydrate metabolism of kidney bean cuttings as affected by treatment with indoleacetic acid. BOT. GAZ. **100**:298–311. 1938.
15. VEGIS, AUSEKLIS, Premature sprouting induced by heteroauxin (indolylacetic acid). Lettonie Biol. Soc. Bull. 7. 87–101. 1937.
16. WENT, F. W., Some experiments on bud growth. Amer. Jour. Bot. **26**:109–117. 1939.
17. ZIMMERMAN, P. W., and HITCHCOCK, A. E., Experiments with vapors and solutions of growth substances. Contrib. Boyce Thompson Inst. **10**: 481–508. 1939.
18. ———, and ———, Formative effects induced with β-naphthoxyacetic acid. Contrib. Boyce Thompson Inst. **12**:1–14. 1941.

SUSCEPTIBILITY OF COLCHICUM AND CHLAMYDOMONAS TO COLCHICINE

IVOR CORNMAN

(WITH EIGHT FIGURES)

Introduction

The striking uniformity with which colchicine checks mitosis and meiosis in higher plants and animals suggests that there is a colchicine-sensitive process common to all mitoses. There are, however, exceptions which cast doubt upon this generalization. *Colchicum* is one of these. Within its tissues are concentrations of colchicine well above those which will stop mitosis in other plants, and its roots can grow in strong colchicine solutions without visible abnormalities (**4**, **19**). Resistance has also been found in numerous protista and fungi, organisms which do not contain colchicine (**25**, **4**, **17**). To study these two types of resistance, *Colchicum* and the green flagellate, *Chlamydomonas*, were chosen. The investigation was directed toward comparing the two types of resistance and particularly toward finding whether the resistance is dependent on the absence of the colchicine-sensitive process. The results obtained with *Colchicum* have been briefly reported previously (**9**).

Material and methods

Roots of *Colchicum byzantinum* were used primarily, with additional observations on the roots of *C. autumnale* for comparison. The roots were cut from the corm when 3–5 cm. long and placed singly or by twos in vials with inside dimensions of 3.5 × 50 mm. Five mm. of the extreme tip of the root was immersed in the experimental or control solution. The upper, cut end of the root was imbedded in 1 per cent agar with 5 per cent glucose, which also served to seal the vial. Thus the isolated roots were protected from drying within sealed vials, and only the mitotically active region was exposed to colchicine. Since a corm of *Colchicum* sometimes produces as many as thirty roots of uniform length, a wide range of concentrations and exposures could be tested on material identical genetically, and—as far as possible—physiologically similar. For the most part, experiments were confined to the afternoon and early evening, although no appreciable diurnal periodicity of mitosis could be detected.

The concentrations of colchicine used were 0.1, 1.0, 5, and 10 per cent by weight. Some question may arise as to the possibility of obtaining a 10 per cent solution, since the U.S. Pharmacopoea X gives the solubility of colchicine as 1 gm. in 22 cc. of water. No difficulty was found in obtaining a 10 per cent solution, however, and HENRY (**14**) lists colchicine as miscible in all proportions with water. Perhaps some of the difficulty in obtaining high concentrations of colchicine results from its conversion in water to the less soluble colchiceine. To avoid such a loss, the solutions were mixed not more than 30 minutes before using and always protected from direct light. No precipitation was observed during the course of the experiments. A few roots were also

exposed to saturated aqueous acenaphthene solution.

Exposures were varied between ½ and 8½ hours. Some treatments were followed by a recovery period in water. Controls were cultured in tap water and in sucrose isomolar with the colchicine. The experiments were conducted at about 22° C. The root tips were fixed in Bouin's or Navashin's solution and stained in iron haematoxylin and eosin. Navashin's is by far the superior fixative for preservation of spindle structure. Observations were made upon a total of about 200 roots of *Colchicum*.

Attempts were also made to test the effect of colchicine on the division of the pollen tube generative cell, using the method by which EIGSTI (10) has obtained such excellent results with other pollens; but *Colchicum* pollen obtained in the region of Ann Arbor would not germinate.

For comparison, and especially as a check on the culture method, excised roots of *Allium cepa* were also grown in vials in the same way as the *Colchicum* roots. The experimental solutions were 0.01 and 0.1 per cent colchicine by weight and saturated aqueous acenaphthene solution. Tap water was used as the control solution. In all, about 100 *Allium* roots were studied.

Bacteria-free cultures of *Chlamydomonas pseudococcus* were obtained from a pure line descended from a single original individual. The culture medium (10 gm. tryptone in 1000 cc. water) contained 0.015, 0.010, or 0.005 per cent colchicine, or acenaphthene to saturation, half saturation, or quarter saturation. The culture tubes were kept in diffuse sunlight, so distributed that no one experimental set had an advantage of light or temperature. Counts of population density were made at the beginning and end of each experiment. The increase was then calculated, both in terms of generations and in terms of percentage of inhibition of the original population. The results presented here are based upon counts of forty-five experimental and control populations.

Observations

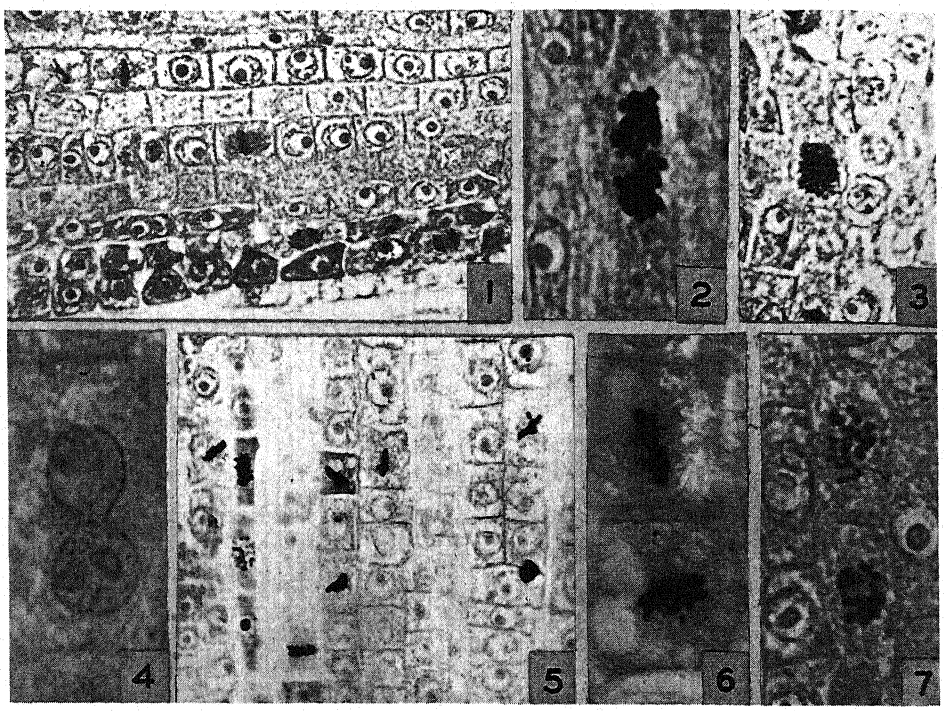
Effect of colchicine on excised roots

Excised root tips cultured in tap water maintained normal mitosis for at least 8½ hours, although the number of mitoses decreased. Similarly, roots cultured continuously in concentrations of sucrose isomolar with the colchicine solutions, or subsequently transferred to tap water, showed normal mitoses throughout the same period. On the contrary, colchicine produced a typical disruption of mitosis, particularly in *Colchicum byzantinum*, to which most of the detailed description will be devoted.

Mitosis in *C. byzantinum* was not altered by 1 per cent colchicine. A 2.5 per cent concentration was near the threshold, producing only a partial effect even after 3 hours' exposure. This concentration, however, can produce tetraploid cells (fig. 3). Five per cent colchicine was the lowest concentration which brought about a complete colchicine effect. During a ½-hour exposure, the structure of existing spindles was destroyed and the organization of new spindles prevented. The metaphase orientation of the chromosomes in figure 1 is evidence of destruction of the spindle after it had already oriented the chromosomes. The disorganized spindle material is still visible in the center cell, showing as an oval area devoid of fibers. This is in marked contrast to the distinct fibers of the normal spindle. In other cells receiving the same treatment meta-

phase chromosomes were irregularly grouped, unable to form an equatorial plate because the spindle had been rendered nonfunctional. A cell in figure 1 (bottom, center) contains four such groups scattered through the darkly staining spindle material. Spindle fibers disappeared from anaphase (fig. 2) and telophase figures also, during ½-hour exposure to 5 per cent colchicine. An anaphase figure with two incompletely separated groups of chromosomes is shown in figure 2. Half-hour exposure did not affect the number of mitoses (table 1).

With longer exposure to 5 per cent colchicine, the spindle material disappeared entirely, and the clumping of chromosomes became more marked. The course of events induced by colchicine is diagrammed in figure 8. The number of



FIGS. 1–7.—Roots of *Colchicum byzantinum* excised and cultured in colchicine solution: Fig. 1, ½-hour exposure to 5 per cent colchicine; central metaphase figure shows disorganized spindle material, lacking fibers; two other metaphases, anaphase, and premetaphase show partial destruction of the spindle. Fig. 2, ½-hour exposure to 5 per cent colchicine; anaphase spindle destroyed before the two chromosome groups had completely separated; such configurations are source of tetraploid nuclei. Fig. 3, 1-hour exposure to 2.5 per cent colchicine; tetraploid cell with chromosomes divided but not separated; cell full of chromosomes; no spindle visible. Fig. 4, 2-hour exposure to 5 per cent colchicine; tetraploid bridged nucleus with four nucleoli (one not visible in photograph); such nucleus results from mitosis checked in anaphase (fig. 3); chromosomes returned to interphase while still exposed to colchicine. Fig. 5, 75-minute exposure to 5 per cent colchicine followed by 105 minutes in tap water; metaphase plates show unusual orientation of spindles (suppressed in photograph to give contrast) which have appeared after removal of root from colchicine. Fig. 6, 1-hour exposure to 5 per cent colchicine followed by 1 hour in tap water; short spindle fibers appear to be extending at right angles from abnormally oriented metaphase plates, as if the spindle were regenerating. Fig. 7, 77-minute exposure to 5 per cent colchicine, followed by 48 minutes in tap water; chromosome clumps produced by colchicine show signs of dispersing; no spindle material remains.

mitoses and the proportions between the phases were changed radically by this longer exposure. In table 1 are recorded the average numbers of mitoses in a block of tissue as wide as the root, extending from the root tip to the proximal border of the meristematic tissue, and 100 μ thick through the center of the root. This tabulation does not allow for the small variations in width of the roots, so that small variations in total numbers of mitoses (such as among the controls for the first 2 hours) probably are not significant. In the roots fixed

TABLE 1

NUMBERS OF MITOTIC STAGES IN CONTROL AND COLCHICINE-TREATED ROOTS OF COLCHICUM BYZANTINUM*

Treatment		Colchicine (5%)					Tap water			
Experiment	Exposure (hours)	No. of roots	Meta-phase	Ana-phase	Telo-phase	Bi-nucleate	Meta-phase	Ana-phase	Telo-phase	No. of roots
A	0	.....	.....	.....	.....	.....	111.5	52.0	36.5	2
	½	3	95.1	34.3	30.5	0	.....	.....	.....	.....
	1	4	182.0	5.0	0	25.3	103.0	33.0	20.0	2
B	2	4	136.3	0.5	0	21.8	114.5	39.5	29.5	2
	4	4	139.8	0.0	0	11.3	37.0	14.0	9.0	1
	8½	3	55.0	0.0	0	10.0	17.7	2.7	4.3	3

*Each number is average per root in block of tissue extending entire length of meristematic region, as wide as root and 100 μ thick, through its center. Under metaphase, control, are included premetaphase stages (membrane gone, chromosomes thick and short but not oriented) indistinguishable from metaphase stages under colchicine effect. In telophase column, only those cells in which nucleus and cell plate are normal are included. After 1-hour exposure, some cells classed as binucleate have telophase nuclei.

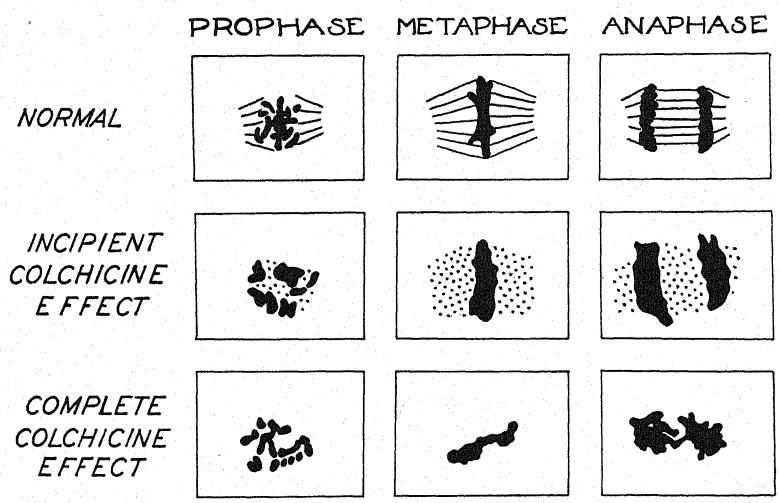


FIG. 8.—Diagram showing sequence of events in different mitotic phases succumbing to colchicine. Spindle fibers, sharply visible in normal cells, disappear, leaving structureless mass of heavily staining spindle material (incipient colchicine effect); then this spindle material also disappears, allowing chromosome groups to drift through cytoplasm (complete colchicine effect). Diagrams patterned after configurations in figs. 1, 2, 6, and 7.

after 1-hour exposure to colchicine, however, the metaphases were double the number found in roots fixed at ½ hour, and the preponderance occurred consistently in all roots from which these counts were averaged. Simultaneously with the accumulation of metaphases there was a decrease in the number of anaphases and telophases. With these changes in numbers of mitotic stages there appeared a new abnormality: binucleate cells, in which the nuclei were separate or connected by a thin bridge. These were almost equal in number to the anaphases which disappeared. Large lobed nuclei with supernumerary nucleoli also appeared at this time.

Prolonged exposure produced a decrease in all mitotic stages, but there was a decrease in the mitotic activity of the controls also (table 1). In some control roots the number of mitoses was normal even 4 hours after cutting, but a decrease always occurred eventually. After 8½ hours of continual exposure, however, normal prophases could still be found in the treated roots, although no other normal mitotic stages were present. The blocked mitoses also decreased in number (table 1). Those which remained became progressively more pycnotic, until at 8½ hours the chromosomes were compacted into a dense knot, usually near the center of the cell.

The course of the colchicine effect appears to be the same, whether it acts upon late prophase, metaphase, or anaphase (fig. 8). There is first destruction of the fibrillar structure of the spindle. Complete disappearance of the spindle material follows, and the chromosomes, singly or in groups, scatter through the cell.

When the roots were allowed to recover in tap water after ½- or 1-hour exposure, the course of events was different from that during continual exposure. The pycnotic clumps of chromosomes loosened and the individual chromosomes became distinguishable again (fig. 7). In some cells which were affected before anaphase the chromosomes were doubled. Other cells, in which mitosis was interrupted after the daughter chromosomes had separated, were demonstrably tetraploid. In some tetraploid cells the chromosomes were partly separated into two irregular diploid sets, producing a configuration similar to that shown in figure 2; in others the chromosomes were scattered (fig. 7). Binucleate cells, bridged nuclei (fig. 4), and lobed nuclei also appeared, as they did in roots given continual exposure. About 1½ hours after removal of the roots from colchicine, spindles reappeared. At first only a few faint fibers were visible (fig. 6), but, judging by the subsequent completion of mitosis, a complete functional spindle finally formed. Such recovering cells give the impression of regeneration of the spindle and continuation of mitosis, rather than of return of the colchicine mitoses to interphase and their replacement by new, partially abnormal mitoses. The chromosomes in figure 6 appear to be arranged in a relic equatorial plate, about which a few short spindle fibers are forming at right angles. WADA (26) has observed regeneration of the spindle in living *Tradescantia* stamen hairs recovering from colchicine. While the appearance of the spindle structure and equatorial plates in recovered cells was normal except for slight irregularities, orientation of the spindles was entirely unusual. Whereas spindles in the proximal part of the meristematic region are nearly always parallel to the axis of the root, the spindles in these recovering cells

took any position (fig. 5). After reappearance of the spindle, the course of mitosis appeared entirely normal.

The effect of colchicine on *C. autumnale* appeared less quickly and was less marked. After 3 hours' exposure to 10 per cent colchicine, the number of mitoses was considerably reduced as compared with the controls. The spindles of some of the remaining mitoses were destroyed, but normal anaphases and telophases were still to be found. The cytological picture after this 3-hour exposure was about the same as in *C. byzantinum* after 3 hours' exposure to 2.5 per cent colchicine. After 6 hours' exposure, destruction of the spindles was complete, leaving only cells with prophase configurations, or pycnotic clumps of chromatin. Lack of material prevented investigation of the recovery processes. Mitosis continued normal in *C. autumnale* during 7 hours' exposure to saturated aqueous acenaphthene.

With regard to the tests with *Allium*, it need merely be stated that mitoses in these excised roots reacted to 0.01 and 0.1 per cent colchicine in a manner similar to that described for intact roots (**12, 9, 18, 21**), except that repeated reduplication of the chromosomes was not obtained. Mitoses are stopped in metaphase, and the chromosomes divide without anaphase separation. Intact *Allium* roots still attached to the bulb were used to test for colchicine in *C. byzantinum* corms. The cells showed a complete colchicine effect after $\frac{1}{2}$-hour exposure to the crushed corm.

Effect of colchicine and acenaphthene on rate of reproduction of Chlamydomonas

Both colchicine and acenaphthene slow the rate of reproduction of *Chlamydomonas pseudococcus*, and the retardation increases as the dose is increased (table 2). Calculated in terms of generations produced by the original population, the maximum inhibition occurred after 50 hours' exposure to 0.015 per cent colchicine, with an average of 2.49 generations, as against 3.64 generations in the control. If, instead of an average effect on the entire population, an all-or-none effect is assumed, with some of the original population completely incapacitated and the others reproducing at the same rate as the controls, the individuals affected by 0.015 per cent colchicine amount to 55.1 per cent, and those affected by 0.005 per cent amount to 40.6 per cent, of the original population. While such a calculation is useful for

TABLE 2

Reproduction of Chlamydomonas pseudococcus in colchicine and acenaphthene

	Colchicine (50 hours)				Colchicine (100 hours)				Acenaphthene (164 hours)	
	Control	0.005	0.010	0.015	Control	0.005	0.010	0.015	Control	Saturated
Generations*	3.64	2.89	2.44	2.49	4.10	4.05	3.41	3.12	5.68	5.05
Difference from control	.....	0.75	1.20	1.15	.....	0.05	0.69	0.98	.....	0.63

* Number of generations calculated as average rate of reproduction for all individuals: initial population $\times 2^n$ = final population, where n = number of generations.

comparison with the root of *Allium*, where 0.01 per cent colchicine blocks mitosis in every cell, it reveals an inconsistency when applied to the results for 100-hour exposure (table 2). On an all-or-none basis, the total population after 100 hours would indicate that only 4.2 per cent of the original population exposed to 0.015 per cent colchicine was completely checked, while 95.8 per cent reproduced at the same rate as the controls. These two different exposures were begun at the same time and seeded with equal quantities of the same stock culture.

The slowing effect of saturated acenaphthene on the rate of reproduction was about the same as the 0.01 per cent colchicine. Half- and quarter-saturated media produced intermediate effects.

Stained preparations of the retarded *Chlamydomonas* revealed no metaphase block or other colchicine effects. It is difficult to observe details within these cells, however, so this cytological evidence is not conclusive.

Discussion

The resistance of organisms to colchicine does not necessarily indicate a difference in the mitotic process. It could also result from some extra-mitotic physiological capacity of the cell to inactivate the colchicine. If the first condition exists, if the mitosis is different from the mitosis of susceptible forms in that it is not dependent upon a process disrupted by colchicine, then the spindle should not respond to colchicine treatment under any conditions. On the contrary, if the resistance is made possible by metabolic peculiarities superimposed upon a typical spindle mechanism, it should be possible to deter these processes, or exceed their capacity, and thus inflict colchicine inhibition upon the spindle mechanism. With regard to *Colchicum*, the evidence presented here indicates that the resistance can be overwhelmed and a typical colchicine effect produced.

There can be no doubt that colchicine produces a very definite effect in *Colchicum* roots. It is certain that these effects are produced by the colchicine and not by other procedures in the method, since mitoses in excised control roots proceed normally, and *Allium* roots in culture show typical colchicine effects. Nor can it be said that *C. byzantinum* is not a suitable organism for investigating resistance to colchicine, inasmuch as there is enough colchicine in the corm to block mitosis in *Allium* roots, and the roots of *C. byzantinum* are immune to 1 per cent colchicine in culture medium.

Since the concentrations which did produce effects are so far above those which produce colchicine mitoses in other plants, however, it is doubtful whether the effects produced in *Colchicum* are typical of the highly specific effects peculiar to colchicine, or rather reveal just a general disruption of the cell. On the one hand, high concentrations might interfere with mitosis by virtue of their osmotic activity. However, mitosis was not disrupted in sucrose solutions isomolar with 10 and 5 per cent colchicine. While isomolarity is only an approximation of osmotic activity, the complete lack of response to sugar solutions is strong evidence against the possibility that the effect produced by colchicine solutions is the result of their osmotic pressure. Moreover, the effects of hypertonicity alone, as reported by numerous investigators, do not include a selective destruction of the spindle structure such as observed in these experiments with colchicine. On the other hand, there may be a general poisoning of the cells, making normal mitosis impossible. Con-

sideration of the specific effect of colchicine rules out this possibility fairly conclusively. Six of the most typical and significant effects of colchicine upon plants are:

1. Spindles disappear and no new ones form.
2. Prophases continue, producing an accumulation of colchicine mitoses.
3. Chromosomes scatter and divide without normal anaphase separation.
4. The cell plate does not form.
5. Blocked metaphases and anaphases pass on to interphase.
6. Chromosome doubling without anaphase separation is repeated, resulting in further increase in ploidy.

Each of these conditions, with the exception of the sixth, was observed in *C. byzantinum* exposed to 5 per cent colchicine.

1. The spindle fibers first (fig. 1), and then the entire spindle, disappeared.

2. Cells in which the chromosomes showed no indication of metaphase orientation accumulated upon moderate exposure (table 1).

3. Particularly in roots returned to tap water could the scattering (fig. 7) and doubling of chromosomes be observed.

4. Reconstruction nuclei were seen in binucleate cells where the cell plate was wholly or partly missing.

5. The decrease in colchicine mitoses (disrupted metaphases) during prolonged treatment (table 1) indicates progression of blocked mitoses to the interphase condition. Also, binucleate cells, bridged nuclei (fig. 4), and nuclei with three or four nucleoli were found, which point to an origin from cells stopped during mitosis. Bridged nuclei, each of the pair having the maximum normal complement of two nucleoli, are hard to explain other than by the return to interphase of two partially separated chromosome groups. Having a single membrane, these are true tetraploid nuclei. The number of nucleoli is in itself a good criterion of polyploidy (3). Nuclei of *C. byzantinum* typically have only one or two nucleoli, so nuclei found with three or four were in all probability tetraploid. To guard against confusion of induced with natural binuclearity, counts of binucleate cells (table 1) were limited to longitudinal sections of the region of the ground meristem, a short distance proximal to the extreme tip of the root. In this region no binucleate cells were found in the controls included in table 1, and only three doubtful cases of binuclearity could be found in a total of sixty-nine normal and control *Colchicum* roots, as against the high percentage of binucleate cells in treated roots.

6. Repeated reduplication of chromosomes could not be expected, since the method did not permit prolonged culturing of the excised roots. It was noticed, however, that binucleate cells which presumably resulted from blocked anaphases re-entered the prophase stage during exposure to colchicine.

The resumption of mitoses which were normal except for orientation of the spindle is further evidence that the cells were not permanently injured and that the primary effect was upon the spindle. The decrease in the number of mitoses in *C. autumnale* is a colchicine effect also observed in other angiosperms (22). All the evidence leads to the conclusion that certainly in *C. byzantinum*, and probably in *C. autumnale*, a typical specific inhibition of the spindle results from exposure to colchicine. LEVAN (18) has already demonstrated that acenaphthene will block mitosis in *Colchicum* roots even quicker than in *Allium*. (The present negative results with 7-hour exposure to saturated aqueous acenaphthene solutions are not unexpected, since LEVAN points out that acenaphthene takes at

least 12 hours to produce an effect in *Colchicum*.) Accordingly it seems safe to conclude that the mitotic mechanism of *Colchicum* is like that of other plants in that it is dependent upon some process which is disabled by colchicine and related substances.

It follows, then, that resistance to colchicine is not proof of a difference in the mitotic process or of the absence of mitosis. For example, conclusions (2)—based partly on the immunity of yeast to colchicine—that the budding process in yeast is amitotic must be regarded with some question.

While the demonstration of a colchicine susceptibility in *Colchicum* eliminates the possibility of a qualitative difference in mitosis, the possibility of a quantitative difference remains to be considered. For instance, the existence of a stronger spindle-forming and spindle-maintaining mechanism in *Colchicum* could be assumed. Returning to LEVAN'S observations, it can be seen that such a supposition is unsatisfactory, inasmuch as *Colchicum* was found to be more sensitive to acenaphthene than was *Allium*. This argument is based on the presumption that colchicine and acenaphthene act in the same way. The assumption of a single point of action of these two chemically similar substances follows from the identity of the visible effects they produce. JARETZKY and SCHENK (16) question the identity of the colchicine reaction with that of acenaphthene. Their conclusions are based on differences in relative susceptibilities to colchicine and acenaphthene in different species. They do not take into account the more probable explanation of these differences: permeabilities to these substances markedly different in solubility. The hypothesis which appears best to fit the observations available to date is also the simplest—that colchicine and acenaphthene act in the same way at the same point in the mitotic cycle in all plants, including *Colchicum*.

The means by which *Colchicum* protects itself from colchicine remains unknown. The readiest explanation would be that the colchicine is inactivated by combination or alteration within the cell. Minor changes in the structure of this alkaloid greatly affect its potency (5, 6), although mere crushing in water is sufficient to release colchicine from the corm, and such treatment in itself is not likely to alter any stable precursor of colchicine. Moreover, BLAKESLEE (4) has attempted unsuccessfully to extract some substance from the *Colchicum* plant which would make other plants resistant. Nevertheless the possibility remains that the colchicine is adsorbed upon, or combined with, proteins or other cellular constituents which are not easily extracted. Destruction of the cell possibly brings about the release of colchicine by enzyme action. ALBO (1) found that colchicine does not occur in the nonliving tissues, even in the intact plant. Indeed it is conceivable that just such a combination of the colchicine with the structural elements of the spindle prevents its normal functioning. LEVAN'S finding (19) of an effect of threshold dosages of acenaphthene and colchicine upon the outermost part of the spindle only suggests a direct effect of the agents on the spindle. In immune cells, protective structures or processes presumably function by preventing the colchicine from reaching the spindle substance. The fact that excised roots could be affected by colchicine, while intact roots were not (4, 19), suggests that some metabolic activity, hampered by cutting the roots, maintains the immunity. This is not conclusive, inasmuch as the concentrations used by

BLAKESLEE and LEVAN were below the concentrations found to be effective in these experiments. Cold or some other general metabolic deterrent might well be employed to test for increased susceptibility to colchicine.

In resistant organisms other than *Colchicum* and related genera, colchicine does not occur within the cells, so there is no need to explain an immunity that does not result from destruction of the colchicine. There is some evidence that such a destruction does occur. *Chlamydomonas* may be considered a colchicine-resistant organism, although it is affected by colchicine. Resistance, it must be understood, here refers only to the typical colchicine effects in stopping cell division, not to any general poisonous effect. In *Chlamydomonas*, reproduction continues in concentrations of colchicine which quickly stop cell division in higher plants. Moreover, a decreasing potency of the colchicine upon prolonged exposure can be seen in comparing the difference between the control and experimental populations after 50 and 100 hours (table 2). There is less difference at the end of 100 hours, showing that the flagellates reproduced actually faster than the controls after an initial retardation by the colchicine. Other things being equal, the retardation should be cumulative, producing a progressively larger discrepancy between treated and control cultures. Actually other things are not equal, as is shown by the decrease in the rate of reproduction of the controls, a normal trend in any culture of protozoa. Population density and age of culture must therefore be taken into consideration. Since these factors were not studied, detailed inferences cannot be drawn, but there is at least a suggestion that there is less effect of the colchicine during the second 50 hours. This could be interpreted as an acquired immunity or as actual destruction of the colchicine by *Chlamydomonas*. The figures for the 0.005 per cent colchicine particularly suggest that the protozoa destroyed the colchicine, quickly reducing the chemical to a subliminal concentration.

To test the possibility that the smaller final population is not the result of a general slowing of growth but rather results from destruction of the reproductive activity of part of the original population, as by blocking of mitosis, the percentage of the population so affected can be calculated. The figures so obtained are inconsistent. In the 0.005 per cent colchicine, a 40.6 per cent suppression of the original population would be necessary to account for the decreased reproduction during 50 hours, but only 4.2 per cent for the difference between control and experimental at the end of 100 hours. Possibly some of the inhibited individuals could have recovered and caught up with the others, but it seems more probable that the effect of colchicine is a slowing of the rate of reproduction of all the population, perhaps through an entirely nonmitotic effect. No attempt was made to test the extent of resistance of *Chlamydomonas* to colchicine, because even complete inhibition of reproduction would be inconclusive without cytological confirmation of a metaphase block, and *Chlamydomonas* does not lend itself to critical examination of cytological details.

There is also some evidence of recovery from acenaphthene effect, which emphasizes the possibility of a different sort of immunity from that found in *Colchicum*. Immunity to acenaphthene as well as to colchicine (*Colchicum* is immune only to colchicine) suggests that *Chlamydomonas* is protected from a class of compounds similar to colchicine, rather than

from colchicine specifically. Ability to destroy certain types of chemicals is the simplest explanation of this general immunity.

Another interesting possible cause of colchicine resistance in unicellular organisms should be considered. Mitosis in *Chlamydomonas*, and in all the species found resistant by KING and BEAMS (17) —with the possible exception of *Chilomonas*—is intranuclear. Metaphase and anaphase, the stages susceptible to colchicine treatment, are completed within the nuclear membrane. One discordant note is the report of GAVAUDAN and KOBOZIEFF (13) of colchicine abnormalities in *Chlamydomonas*, but VANDENVRIES and GAVAUDAN (25), using *Chlamydomonas* from the same source the following year, were unable to obtain abnormalities. Tests upon a series such as the closely related *Polytomella*, *Parapolytomella*, and *Polytoma*, in which the nuclear membrane disappears at successively later stages in mitosis, should prove particularly interesting.

Resistance of an organism to alkaloids is not to be regarded as an isolated phenomenon. WERNER (27) rightly emphasizes that plants differ in their susceptibility to colchicine. DRAGONE-TESTI (8) presents evidence that growth of *Chlamydomonas* is actually improved by several alkaloids, although her data are not quantitative. Yeast also responds favorably to colchicine (23) and other alkaloids (11). LUTZ (20) showed that numerous plants grew better when alkaloids were added to the nutritive medium and that the alkaloids disappear from the medium. RICHARDS (23) found that colchicine can partly replace the usual sources of nitrogen for yeast. There is in HIGBEE's report (15) a suggestion that even vertebrates are able to metabolize colchicine. Chicks injected with it responded as if to male sex hormone, evidenced by stimulation of comb growth. This increase in cell proliferation may not have been a direct effect of the colchicine, however, although its steroid structure makes such a direct action upon the comb reasonable to expect.

These cases differ from the immunity of *Colchicum* in that the colchicine is presumably destroyed and utilized in some other form. They are important in that they emphasize the general occurrence in organisms of processes which can alter or restrain alkaloids before any deleterious effect can be exerted. Until cytological verification of a colchicine effect upon additional resistant forms shows that the spindle is susceptible, it cannot be assumed that the mechanism of resistance is extra-mitotic. The resistance of yeast appears to be of this sort (24), and the available evidence at least leaves open the possibility that colchicine resistance in general depends upon metabolic processes not directly concerned with spindle formation, as appears to be the case in *Colchicum*.

In conclusion, it can be said that *Colchicum*, while resistant to concentrations of colchicine 100 times those which completely block mitosis in other angiosperms, responds to high concentrations with a typical colchicine effect. Accordingly, the immunity of *Colchicum* resides not in a difference in the mitotic process but in some extra-mitotic protective agency. This resistance appears to be specific for colchicine. *Chlamydomonas*, resistant to acenaphthene as well as to colchicine, may possess a more general protective agency by which it escapes or inactivates any substances chemically similar to colchicine. This more general type of immunity may be the basis of resistance of organisms not related to *Colchicum*.

Summary

1. Excised roots of *Colchicum* cultured in small vials are immune to 1 per cent colchicine.

2. Mitosis in excised roots of *C. byzantinum* can be partly disrupted by 2.5 per cent colchicine and entirely blocked by 5 per cent. Mitosis in *C. autumnale* is first disrupted and eventually completely blocked by 10 per cent colchicine. The effects are typical of those produced in other angiosperms.

3. It is concluded that the immunity of *Colchicum* results from an extramitotic protection, not from a difference in the mitotic mechanism.

4. *Chlamydomonas pseudococcus*, as judged by the rate of reproduction, is resistant both to colchicine and acenaphthene. This immunity is not specific for colchicine and may represent the type of resistance found in organisms which do not naturally contain colchicine.

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LITERATURE CITED

1. ALBO, G., Sur la signification physiologique de la colchicine dans les différentes espèces de *Colchicum* et de *Merendera*. Arch. Sci. phys. nat. **12**: 227–236. 1901.
2. BEAMS, H. W., ZELL, L. W., and SULKIN, N.M., Cytological studies on yeast cells with special reference to the budding process. Cytologia **11**: 30–36. 1940.
3. BERGER, C. A., Some criteria for judging the degree of polyploidy of cells in the resting stage. Amer. Nat. **75**:93–96. 1941.
4. BLAKESLEE, A. F., The present and potential service of chemistry to plant breeding. Amer. Jour. Bot. **26**:163–172. 1939.
5. BLAKESLEE, A. F., *et al.*, Chromosome investigations. Carneg. Inst. Wash. Year Book **37**:35–40. 1938.
6. BRUES, A. M., and COHEN, A., Effects of colchicine and related substances on cell division. Biochem. Jour. **30**:1363–1368. 1936.
7. CORNMAN, I., Disruption of mitosis in *Colchicum* by means of colchicine. Biol. Bull. **81**:297–298. 1941.
8. DRAGONE-TESTI, G., Azione di alcuni alcaloidi sullo sviluppo di *Chlamydomonas* e di *Euglena*. Ann. di Bot. **20**:38–43. 1933.
9. EIGSTI, O. J., A cytological study of colchicine effects in the induction of polyploidy in plants. Proc. Nat. Acad. Sci. **24**:56–63. 1938.
10. ———, The effects of colchicine upon the division of the generative cell in *Polygonatum*, *Tradescantia*, and *Lilium*. Amer. Jour. Bot. **27**:512–524. 1940.
11. ENDERS, C., and WIENINGER, F. M., Die Beeinflussung des Vergärungs- und Vermehrungsvermögens der Hefe durch Alkaloide. Biochem. Zeitschr. **293**:22–29. 1937.
12. GAVAUDAN, P., GAVAUDAN, N., and POMRI-ASKINSKY-KOBOZIEFF, N., Sur l'influence de la colchicine sur la caryocinèse dans les méristèmes radiculaires de l'*Allium cepa*. Compt. rend. Soc. Biol. **125**:705–708. 1937.
13. GAVAUDAN, P., and KOBOZIEFF, N., Action de la colchicine sur la caryocinèse et la cytodiérèse des Chlamydomonadinées. Compt. rend. Soc. Biol. **127**:790–793. 1938.
14. HENRY, T. A., The plant alkaloids. 3d ed. Blakiston. 1939.
15. HIGBEE, E., Further effects of colchicine on developing chicks (Abstract). Anat. Rec. **78**: suppl. p. 48. 1940.
16. JARETZKY, R., and SCHENK, G., Versuche mit Acenaphten und Colchicin an Gramineen und Leguminosenkeimlingen. Jahrb. wiss. Bot. **89**: 13–19. 1940.
17. KING, R. L., and BEAMS, H. W., A comparison of the effects of colchicine on division in protozoa and certain other cells. Jour. Cell. Comp. Physiol. **15**:252–254. 1940.
18. LEVAN, A., The effect of colchicine on root mitoses in *Allium*. Hereditas **24**:471–486. 1938.
19. ———, The effect of acenaphthene and colchicine on mitosis of *Allium* and *Colchicum*. Hereditas **26**:262–276. 1940.
20. LUTZ, M. L., Recherches sur la nutrition des

végétaux a l'aide des substances azotées de nature organique. Ann. Sci. nat. Bot. Ser. 8. 7:1–103. 1898.

21. MANGENOT, G., Effet de la colchicine sur la mitose dans les racines d'*Allium cepa* et d'*Hyacinthus orientalis*. Compt. rend. Soc. Biol. 128: 501–504. 1938.
22. ———, L'action de la colchicine sur les cellules végétales. Compt. rend. Acad. Sci. 208:222–224. 1939.
23. RICHARDS, O. W., Colchicine stimulation of yeast growth fails to reveal mitosis. Jour. Bacter. 36:187–195. 1938.
24. SINOTÔ, Y., and YUASA, A., Karyological studies in *Saccharomyces cerevisiae*. Cytologia 11:464–472. 1941.
25. VANDENVRIES, R., and GAVAUDAN, P., Action de la colchicine sur quelques organismes inférieurs. Compt. rend. Acad. Sci. 208: 1675–1677. 1939.
26. WADA, B., Lebendbeobachtungen über die Einwirkung des Colchicins auf die Mitose, insbesondere über die Frage der Spindelfigur. Cytologia 11:93–116. 1940.
27. WERNER, G., Zytologische Untersuchungen über die Wirkun des Colchicins bei zwei verschieden reagierenden Pflanzen: Lein und Erbse. Biol. Zentralbl. 60:86–103. 1940.

BIOCHEMICAL NITROGEN FIXATION STUDIES. IV. EXPERIMENTS WITH EXCISED LEGUME NODULES

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Nitrogen fixation in leguminous plants is a symbiotic process which takes place in the root nodule and accompanies growth, although the rates at which growth and fixation occur are not necessarily parallel. Numerous attempts have been made to secure fixation with the bacteria alone or with uninoculated legumes, but there is no convincing evidence that either of the symbionts alone possesses this ability. A few workers have attacked the problem of symbiotic nitrogen fixation by removing the nodules from the plant and determining the uptake of nitrogen by whole or crushed nodules in the presence of various substrates.

KRASCHÉNINNIKOV (6) seems to have been the first to report nitrogen fixation studies with excised nodules. Six- to 38-gm. samples of lupine and bean nodules alone, or attached to the roots, were maintained in a nitrogen-oxygen mixture, and at the end of 24–93 hours the decrease in nitrogen was measured. Five results with whole roots having nodules attached showed fixations of 0.17–1.68 ml. nitrogen (average 0.69 ml.); nineteen results with nodules alone gave results varying from −0.33 to 2.27 ml. nitrogen (average 0.49 ml.) fixed. He concluded that if adequate oxygen is supplied to permit intensive respiration, nitrogen fixation can be observed, although it is small.

BEIJERINCK (4) experimented with 10–1000-gm. samples of nodules of yellow lupine, serradella, broad bean, and locust maintained at 25° C. in air in wide glass tubes for periods up to 20 days. Nitrogen changes, measured gasometrically, showed no fixation in any case. WILSON, HOPKINS, and FRED (13) kept small samples (0.4–0.8 gm.) of pea nodules for 22–30 days, either in a flask supported on glass beads above a nutrient solution or in a well-aerated tower where they were bathed with a mannitol-phosphate nutrient solution at intervals. Analyses for nitrogen by the Kjeldahl method showed no fixation. GALESTIN (5) likewise observed no nitrogen fixation in five experiments in which 345–510 gm. of nodules of yellow lupine were maintained for 2–3 days in a special apparatus designed to allow gas circulation. Nitrogen changes were measured gasometrically.

It may be noted that when WINOGRADSKY (14) presented his hypothesis that the ammonia evolved from excised nodules is a direct product of nitrogen fixation, he assumed that such nodules could fix nitrogen. In the absence of proof of fixation, his arguments obviously carry little weight.

Recently VIRTANEN (9) and VIRTANEN and LAINE (10, 11) reported nitrogen fixation with whole or crushed pea nodules kept in a medium containing oxalacetic acid, but not with glucose alone as the substrate. Nitrogen fixation was demonstrated both by the Kjeldahl method and by decrease in the volume of nitrogen gas. They (11) state that distinct nitrogen fixation occurred within a

few hours in all cases where neutral oxalacetic acid was present. The results of the one experiment reported in detail show a 10 per cent increase in nitrogen in 24 hours. Attempts to duplicate VIRTANEN'S results with excised pea nodules have given negative results (15, 12). In fact, about 75 per cent of the oxalacetic acid was decomposed by the crushed nodules within an hour, and practically all had disappeared within 3 hours.

The present paper gives the results of nitrogen fixation experiments with whole and crushed nodules, as well as with extracted juices, using several experimental methods and plant species. Brief reference (1) has already been made to these studies, nearly all of which were completed prior to 1937.

Methods

The procedure in most of the experimental work consisted in placing freshly gathered and thoroughly washed nodules in a mineral-sugar culture solution (2) in Warburg vessels and analyzing for decrease in volume of nitrogen gas after various periods. The manometers attached to the vessels were filled with mercury instead of with the usual Brodie's solution, since the gas changes due to respiration were comparatively large. The nodules were kept in various gas mixtures at a temperature of 28° C. and constantly shaken throughout the experiment. They were not sterilized except in a few cases (noted in the tables). The depth of culture medium (usually 2 ml. per vessel) was such that ordinarily about half the larger nodules were immersed and the other half exposed to the atmosphere. In a few experiments nodules were placed in a liquid culture soution in Erlenmeyer flasks or in large Petri dishes at various temperatures, where they were only partially submerged, and the nitrogen uptake measured by determination of total nitrogen at the beginning and end of the experiment. Most of the details of the procedure followed have already been described (2).

Three methods of measuring the gain or loss of nitrogen by the nodules were used.

1. In the experiments reported in table 1 the gas was removed from each Warburg vessel at the end of the experiment by immersing the vessels (still attached to the manometers) in mercury, removing the glass stopper, and catching the gas displaced in a test tube. The percentage composition of this sample, as well as that of the gas mixture originally introduced, was determined by the gas analysis method (8). The volumes of gas at normal temperature and pressure originally and at the end of the experiment were calculated from the vessel constants and the manometer readings. In this way the increase or decrease in nitrogen was readily calculated.

2. In the experiments reported in table 2 the changes in nitrogen were measured in Warburg respirometers. The nitrogen present at the beginning was calculated from the volume of the vessel and either the known composition of air or manometric data on blank vessels from which the oxygen had been absorbed. That present at the end of the experiment was calculated from manometric data after absorption of the oxygen, which was accomplished by pyrogallol in a side-arm into which alkali was tipped from an adjacent side-arm. Since acid pyrogallol does not absorb oxygen, its consumption could be measured in the usual way. Final manometric readings were made 20 minutes after addition of the alkali to the pyrogallol.

3. In the experiments reported in

TABLE 1

NITROGEN FIXATION BY YOUNG COWPEA NODULES MAINTAINED IN WARBURG RESPIRATION VESSELS

Material	Weight (gm.)	O_2 in N_2 used (%)	Glucose (%)	Time (hours)	Gas volume in ml. (N.T.P.)		
					Initial N_2	Final N_2	N_2 fixed
Experiment 1:							
Nodules, whole	1	49.8	3*	22	9.37	9.89	−0.52
Nodules, whole	1	49.8	3*	22	9.39	9.91	−0.52
Nodules, whole	1	49.8	3*	22	9.21	9.51	−0.30
Nodules, whole	1	49.8	3*	22	9.30	9.37	−0.07
Nodules, whole	1	49.8	3*	22	9.60	9.91	−0.31
Nodules, whole	1	49.8	3*	22	9.69	9.81	−0.12
Nodules, whole†	1	49.8	3*	22	8.97	9.47	−0.50
Nodules, whole	1	49.8	0	22	9.43	9.76	−0.33
Experiment 2:							
Nodules, crushed	1	49.8	0	9	8.44	8.72	−0.28
Nodules, crushed	1	49.8	1	9	8.76	8.99	−0.23
Nodules, crushed	1	49.8	1	9	8.84	8.93	−0.09
Nodules, whole	1	49.8	0	9	8.90	9.08	−0.18
Nodules, whole	1	49.8	0	9	8.69	8.88	−0.19
Nodules, whole	1	49.8	1	9	8.81	9.41	−0.60
Nodules, whole	1	49.8	1	9	8.62	8.95	−0.33
Nodules, crushed‡	1	49.8	1	9	8.35	8.76	−0.41
Nodules, whole‡	1	49.8	1	9	8.57	8.71	−0.14
Roots	1	49.8	1	9	8.13	8.30	−0.17
Roots	1	49.8	1	9	8.58	8.88	−0.30
Experiment 3:							
Nodules, crushed	1	49.8	1	13	9.42	9.75	−0.33
Nodules, crushed	1	49.8	1	13	9.29	9.58	−0.29
Nodules, crushed	1	49.8	1	28	9.30	9.35	−0.05
Nodules, crushed	1	49.8	1	28	9.81	9.80	0.01
Roots	1	49.8	1	28	9.84	10.14	−0.30
Nodules, crushed	1	0	0	50	18.38	18.47	−0.09
Nodules, crushed	1	0	1	50	18.01	17.77	0.24
Roots	1	0	1	50	17.76	18.00	−0.24
Experiment 4:							
Nodules, whole§	1	0	1	44	17.36	17.23	0.13
Nodules, whole§	1	0	1	68	15.79	14.48	1.31
Nodules, crushed§	1	0	1	44	17.10	17.05	0.05
Nodules, crushed§	1	0	1	68	16.73	16.65	0.08
Roots	1	0	1	44	17.59	18.65	−1.06
Roots	1	0	1	68	16.88	17.93	−1.05
Nodules, crushed	1	0	0	44	16.94	16.85	0.09
Nodules, crushed	1	0	1	44	16.76	16.64	0.12
Nodules, crushed	1	0	1	44	16.00	15.85	0.15
Nodules, crushed	0.5	0	1	44	17.56	17.48	0.08
Nodules, crushed	0.5	0	1	44	16.01	16.01	0.00

* Sucrose substituted for glucose.

† Sterilized 1 minute in 90% alcohol and then washed.

‡ These vessels contained 0.2 ml. of 1:100 $HgCl_2$ solution.

§ Nodules and vessels sterilized for 3 minutes in acetone before using.

TABLE 1—*Continued*

Material	Weight (gm.)	O_2 in N_2 used (%)	Glucose (%)	Time (hours)	Gas volume in ml. (N.T.P.)		
					Initial N_2	Final N_2	N_2 fixed
Experiment 5:							
Nodules, dried	0.2	21	3	20	13.92	14.19	−0.27
Nodules, dried	0.2	21	3	45	13.72	13.96	−0.24
Nodules, dried	0.2	21	3	45	13.43	13.67	−0.24
Nodules, dried	0.2	5	3	45	16.04	16.52	−0.48
Nodules, dried	0.075	0	3	45	17.21	17.10	0.11
Nodules, dried	0.075	0	3	45	16.62	16.28	0.34
Experiment 6:							
Nodules, whole	1	21	3	44	12.44	12.83	−0.39
Nodules, whole	1	21	3	44	13.40	13.35	0.05
Nodules, whole	2	21	3	26	12.45	12.53	−0.08
Nodules, whole	2	21	3	26	12.19	12.23	−0.04
Nodules, crushed	2	21	3	19	11.62	11.64	−0.02
Nodules, whole	2	21‖	3	26	12.11	12.01	0.10
Nodules, whole	2	21‖	3	26	12.44	12.41	0.03
Nodules, crushed	2	21‖	3	19	12.32	12.23	0.09
Experiment 7:							
Nodules, whole	1	21	3	29	14.62	14.61	0.01
Nodules, whole	1	21	3	29	15.07	15.10	−0.03
Nodules, whole	1	21‖	3	29	13.70	13.72	−0.02
Nodules, whole	1	21‖	3	29	13.72	13.62	0.10
Nodules, whole	1	0	3	19	17.93	17.71	0.22
Nodules, whole	1	0	3	19	18.19	18.10	0.09
Roots	1	21	3	42	13.90	13.96	−0.06
Roots	1	21	3	42	14.93	14.93	0
Nodules, whole	0.2	21	3	42	15.32	15.32	0

‖ Additional oxygen added at intervals to replace that consumed in respiration.

table 3 nitrogen fixation was measured by the Kjeldahl method.

Experimental results

PRELIMINARY EXPERIMENTS USING EXCISED SWEET CLOVER NODULES.—Young sweet clover nodules, either whole or crushed, were maintained in Warburg respiration vessels for 24 hours in a mineral solution containing 3 per cent sucrose. The original gas mixture consisted of 49.8 per cent oxygen in nitrogen, but most of the oxygen was consumed within 12–15 hours. Additional oxygen was not added during the experiment. The gas analyses, not reported in detail, gave no evidence that appreciable nitrogen fixation occurred.

ANALYSES BY SHEPHERD GAS ANALYSIS METHOD USING EXCISED COWPEA NODULES.—In these experiments a number of variables were tested (table 1), including oxygen and nitrogen percentages, glucose concentration, and the effect of crushing. Roots were used as a control.

In only one vessel was there fixation of as much as 0.5 mg. nitrogen, and in this case a similarly treated vessel, except for a shorter incubation period, showed no significant decrease in volume of the gas. A summary of the analytical results gives no indication of nitrogen fixation. The tendency for a slight increase in nitrogen in the various flasks can undoubtedly be attributed to experimental error, since a careful study of all the possible errors showed that most of these would tend to give negative

rather than positive nitrogen fixation. Transfer of the gas samples from the Warburg vessels to the analytical apparatus was accomplished with some difficulty, and the chances of error in this operation were greater than in the subsequent analysis.

ANALYSES MADE DIRECTLY IN WARBURG VESSELS USING CROWN VETCH AND SOYBEAN NODULES.—In these experiments air was used as the initial gas, and final analysis was made before the manometer readings indicated that the oxygen was all used up (table 2). This

TABLE 2

NITROGEN FIXATION BY YOUNG CROWN VETCH AND SOYBEAN NODULES MAINTAINED IN WARBURG RESPIRATION VESSELS

MATERIAL*	WEIGHT (GM.)	TIME (HOURS)	GAS VOLUME IN ML. (N.T.P.)		
			INITIAL N_2	FINAL N_2	N_2 FIXED
Experiment 8:					
Vetch nodules, whole	1.5	6	13.91	13.90	0.01
Vetch nodules, whole	1.5	6	14.46	14.45	0.01
Vetch nodules, whole	1	6	13.44	13.37	0.07
Vetch nodules, whole	1	6	14.78	14.76	0.02
Experiment 9:					
Soybean nodules, whole	1	0	10.89	10.91	−0.02
Soybean nodules, whole	1	0	12.69	12.71	−0.02
Soybean nodules, whole	1.5	10	12.71	12.65	0.06
Soybean nodules, whole	1.5	10	12.67	12.65	0.02
Soybean nodules, whole	1	10	12.70	12.68	0.02
Soybean nodules, whole	1	10	12.86	12.82	0.04
Soybean roots	2	10	13.35	13.36	−0.01
Blank	0	10	14.74	14.73	0.01
Experiment 10:					
Soybean nodules, whole	1	0	13.67	13.66	0.01
Soybean nodules, whole	1	11	16.15	16.17	−0.02
Soybean nodules, whole	1	11	13.83	13.78	0.05
Soybean nodules, whole	2	11	13.86	13.91	−0.05
Soybean nodules, whole	2	11	12.56	12.52	0.04
Soybean roots	2	11	12.78	12.82	−0.04
Blank	0	11	14.88	14.88	0.00
Experiment 11:					
Vetch nodules, whole	1	2	13.11	13.16	−0.05
Vetch nodules, whole	1	2	10.53	10.57	−0.04
Vetch nodules, whole	1	9.3	11.39	11.35	0.04
Vetch nodules, whole	2	7	10.44	10.43	0.01
Vetch nodules, whole	2	7	10.80	10.78	0.02
Blank	0	9.3	12.03	12.01	0.02
Experiment 12:					
Soybean nodules, whole	2	8.5	11.59	11.60	−0.01
Soybean nodules, whole†	2	8.5	11.78	11.76	0.02
Soybean nodules, whole	2	8.5	13.33	13.36	−0.03
Soybean nodules, whole†	2	8.5	11.84	11.88	−0.04
Soybean nodules, whole†	2	8.5	11.87	11.86	0.01
Soybean nodules, whole†	2	8.5	11.32	11.35	−0.03
Blank	0	8.5	15.39	15.44	−0.05

* All plant material maintained in 21% O_2 in N_2 and in usual basal medium containing 2% glucose.
† These vessels had 0.3 ml. $KMnNO_4$ in side-arms for absorption of any hydrogen gas produced.

prevented the evolution of hydrogen gas, which earlier experiments had shown is given off under anaerobic conditions. Any hydrogen evolved would not be absorbed by alkaline pyrogallol, of course, and would obscure any fixation that might have taken place.

The extreme accuracy of this method is due in large part to its simplicity, particularly since it is unnecessary to transfer the gas sample. So long as the only gases involved are nitrogen, oxygen, and carbon dioxide, the method is very satisfactory. An average of all the thirty-two analyses reported in table 2 shows extreme variations, ranging between +0.09 and −0.06 mg. nitrogen. Certainly there is no evidence that whole or crushed nodules maintained in air in a 2 per cent glucose solution can fix atmospheric nitrogen.

ANALYSES FOR TOTAL NITROGEN BY KJELDAHL METHOD USING COWPEA NODULES KEPT IN ERLENMEYER FLASKS AND PETRI DISHES.—In table 3 are given the results of one series of experiments run in Erlenmeyer flasks and one in Petri dishes. In the Erlenmeyer experiment the degree of aeration was varied, particularly with the idea of keeping the oxygen concentration below that in air, since the inner tissues of ordinary nodules are partially anaerobic (2). In the experiment with Petri dishes the nodules were kept for 24–72 hours at various temperatures to determine whether the nitrogen fixed would be greater under conditions where nodule disintegration was markedly slowed down.

The analyses show variations between +0.57 and −0.10 mg. nitrogen fixed for each 1- or 2-gm. sample. While the average shows a slight fixation, the quantity is so small as to be almost negligible. The fixations of 0.22–0.57 mg. nitrogen obtained at 4°–12° C. may be barely significant.

NITROGEN FIXATION IN PRESENCE OF OXALACETIC ACID.—As already stated,

TABLE 3

NITROGEN FIXATION BY YOUNG COWPEA NODULES MAINTAINED IN NUTRIENT SOLUTION IN ERLENMEYER FLASKS OR PETRI DISHES

MATERIAL (NODULES) AND TREATMENT	TIME (HOURS)	NITROGEN (MG.)		
		TOTAL	AVERAGE	FIXED
Experiment 13: Erlenmeyer flasks*				
Untreated	0	9.6	.....	.....
Untreated	0	9.4	.....	.....
Untreated	0	9.5	.....	.....
Untreated	0	9.7	9.55	.....
Aerated with air	24	9.6	.....	.....
Aerated with air	24	9.3	9.45	−0.10
Aerated with 2% O_2 in N_2	24	10.2	.....	.....
Aerated with 2% O_2 in N_2	24	9.3	.....	.....
Aerated with 2% O_2 in N_2	24	9.2	9.57	0.02
Aerated with N_2	24	9.5	.....	.....
Aerated with N_2	24	10.4	.....	.....
Aerated with N_2	24	9.7	.....	.....
Aerated with N_2	24	9.6	9.80	0.25
In cotton-stoppered flasks	24	9.5	.....	.....
In cotton-stoppered flasks	24	9.8	.....	.....
In cotton-stoppered flasks	24	9.5	.....	.....
In cotton-stoppered flasks	24	10.0	9.70	0.15
Experiment 14: Petri dishes†				
Untreated	0	18.9	.....	.....
Untreated	0	19.3	.....	.....
Untreated	0	18.8	.....	.....
Untreated	0	19.3	19.08	.....
Kept at 28° C.	24	19.2	.....	.....
Kept at 28° C.	24	19.3	.....	.....
Kept at 28° C.	24	19.1	.....	.....
Kept at 28° C.	24	18.9	19.13	0.05
Kept at 12° C.	24	19.5	.....	.....
Kept at 12° C.	24	19.9	.....	.....
Kept at 12° C.	24	19.4	.....	.....
Kept at 12° C.	24	18.8	19.40	0.32
Kept at 4° C.	24	19.9	.....	.....
Kept at 4° C.	24	20.0	.....	.....
Kept at 4° C.	24	19.7	.....	.....
Kept at 4° C.	24	19.0	19.65	0.57
Kept at 12° C.	72	19.7	.....	.....
Kept at 12° C.	72	19.4	19.55	0.47
Kept at 4° C.	72	19.6	.....	.....
Kept at 4° C.	72	19.0	19.30	0.22

* One gm. of nodules incubated in 50 ml. of mineral medium containing 2% mannite and 1% glucose.

† Two gm. of nodules incubated in air in 25 ml. of mineral-mannite-glucose medium.

marked increases have been reported in the nitrogen content of pea nodules kept in a medium containing neutralized oxalacetic acid (9, 10, 11). The writers had hoped to check this finding thoroughly but have not as yet had an opportunity to do so. A few preliminary experiments using cowpea and peanut

nodules gave inconclusive results, but the nodules used were from rather mature plants. Incidentally, pure cultures of rhizobia supplied with oxalacetic acid also fixed no nitrogen. Evidently the claims as to the importance of oxalacetic acid in the nitrogen fixation process, as it occurs in the nodule, will need confirmation, especially in view of some negative results (12, 15).

NITROGEN FIXATION WITH ENZYMES EXTRACTED FROM NODULES.—BACH, YERMOLIEVA, and STEPANIAN (3) reported that the juices pressed from *Azotobacter* and added to a phosphate buffer solution containing mannite or glucose fixed as high as 276 mg. nitrogen per gram of glucose consumed, of which 223 mg. was ammonia nitrogen. ROBERG (7) carried on similar experiments but in no case found evidence of nitrogen fixation by *Azotobacter* juices treated in a variety of ways.

Shortly after BACH and co-workers published their paper, a series of fifteen experiments, each involving a number of variables, was conducted in this laboratory using the juices of legume nodules instead of that from *Azotobacter*. In general, the investigations were conducted in the same manner as those carried out by the Russian workers. Hairy vetch or cowpea nodules were usually ground in sand and celite and pressed in a hydraulic press at 5,000–20,000 pounds per square inch and the juices added in various proportions to a phosphate buffer solution (200 mg. K_2HPO_4 per liter), maintained in thin layers in Erlenmeyer flasks. Glucose or mannitol was supplied to most of the cultures. The juices were usually sterilized by passing through an L_3 filter candle, but in a few cases pasteurization at 60° C. for periods of 20 minutes to 2 hours was used. In some cases unsterilized juices and press cake were also added to the sugar-phosphate culture medium. Most experiments were conducted at 28° C., although a few of the cultures were kept at 10° C. The pH was varied between 5.5 and 7.5. The incubation period was 2–9 days. In some tests the enzyme solutions were aerated with air and provision made for catching any ammonia that might be evolved. Micro-Kjeldahl analyses for nitrogen were made on the freshly pressed juice and on the juice plus culture medium at the end of the experiment. A few nitrogen fixation determinations were made in Warburg respiration vessels using pyrogallol to absorb the gas, as already described in connection with studies of whole nodules. There was no indication that the enzymes extracted from nodules possess the power to fix nitrogen apart from the living cell.

Discussion

The results reported here are in agreement with previous data in showing that excised nodules, even though supplied with glucose, sucrose, or mannite, do not fix appreciable quantities of atmospheric nitrogen. The fixation at 4°–12° C. of 0.22–0.57 mg. nitrogen per 2-gm. sample, as shown by Kjeldahl analysis, may be barely significant but represents at most only a 3 per cent gain over the controls. In all experiments where analyses were made by gasometric methods there was a slight tendency toward negative rather than positive fixation. The results given in table 2, which are the most accurate of those reported here, definitely show no fixation. Incidentally, the number of negative results obtained with unsterilized nodules, maintained in a glucose-mineral medium, shows that *Azotobacter* is not a serious factor in experiments

such as these, at least not unless the incubation period is longer than 24 hours.

These studies also show that the direct gasometric determination of nitrogen fixation in Warburg vessels or similar apparatus, according to the method given, is a satisfactory way of measuring nitrogen gas changes in plant material such as nodules, provided hydrogen or gases other than carbon dioxide are known not to be evolved. One objection to the method, however, is that the rate of respiration of nodules is such that usually most of the oxygen in the ordinary Warburg vessel is consumed within about 12 hours, using a 1-gm. sample of nodules, and of course proportionately sooner with larger samples. For most experiments it would be better to use a 5- or 10-gm. sample and continue the experiment for about 24 hours. This would necessitate the use of vessels other than the usual Warburg ones, or else oxygen—which unfortunately is seldom free from impurities—would have to be added at rather frequent intervals. If anaerobic conditions develop, hydrogen is usually evolved and the method is unsatisfactory.

In general, these experiments would seem to indicate that legume nodules ordinarily either cease fixing atmospheric nitrogen when excised or else fixation continues at such a slow rate that it cannot be measured accurately. Since nodules decompose quickly after being removed from the plant, and since the rate of fixation when attached to the plant is known to be more or less proportional to the rate of growth of the plant, it is not surprising that the quantity of nitrogen fixed by nongrowing excised nodules is at best small. These studies further suggest that in future nitrogen fixation experiments with excised nodules reliance should be placed on gasometric methods of analysis, using larger samples of nodules than customarily used in the present work. There is no indication that an incubation period longer than 1–2 days would be advantageous, unless the work is done at low temperatures or under other conditions where nodule disintegration is less rapid than under ordinary conditions.

Summary

Experiments with excised nodules of sweet clover, cowpea, crown vetch, soybean, and hairy vetch attempted to determine whether such nodules possess the ability to fix atmospheric nitrogen when maintained in culture solutions in the laboratory. Nitrogen fixation was determined by a standard gas analysis method, as well as directly in Warburg respiration vessels and also by Kjeldahl analysis for total nitrogen. The results were as follows:

1. No nitrogen fixation was observed with whole or crushed nodules kept in Warburg vessels at 28° C. in various gas mixtures and in a mineral-culture solution containing sucrose, glucose, or mannite.

2. A 1–3 per cent increase in nitrogen, which is scarcely significant, was obtained in a few cases where nodules were kept in thin layers of a mannite-glucose medium at 4°–12° C. for 1–3 days.

3. Pure cultures of rhizobia did not fix nitrogen when supplied with oxalacetic acid. A few similar experiments with excised nodules gave negative or inconclusive results.

4. Fifteen series of experiments, in which many variables were considered, gave no evidence that the juices extracted from nodules and sterilized by

passing through filter candles can fix atmospheric nitrogen when incubated in mineral-sugar solutions. These results agree with those obtained in similar studies conducted by ROBERG using *Azotobacter* juices, but not with those of BACH, who reported nitrogen fixation with similar material.

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LITERATURE CITED

1. ALLISON, F. E., LUDWIG, C. A., HOOVER, S. R., and MINOR, F. W., Legume nodule metabolism and nitrogen fixation. Nature **144**:711–712. 1939.
2. ———, Biochemical nitrogen fixation studies. I. Evidence for limited oxygen supply within the nodule. BOT. GAZ. **101**:513–533. 1940.
3. BACH, A. N., YERMOLIEVA, Z. V., and STEPANIAN, M. P., The fixation of atmospheric nitrogen by the agency of enzymes extracted from cultures of *Azotobacter chroococcum*. Compt. rend. Acad. Sci. n.s. U.R.S.S. **1**:22–24. 1934.
4. BEIJERINCK, M. W., The significance of the tubercle bacteria of the Papilionaceae for the host plant. Proc. Sect. Sci. Kon. Akad. Wetensch. Amsterdam **21**:183–192. 1918.
5. GALESTIN, G. J. A., Is elementary nitrogen absorbed by nodules on the roots of Leguminosae in the assimilation of atmospheric nitrogen? Chem. Weekblad **30**:207–209. 1933.
6. KRASCHÉNINNIKOV, TH., Assimilation of nitrogen gas by the root nodules of leguminous plants. (In Russian with French summary.) Recueil d'articles scientifiques dédié au Prof. CLÉMENT TIMIRIAZEFF, Moscow. 307–324. 1916.
7. ROBERG, M., Beiträge zur Biologie von Azotobakter. III. Zur Frage eines ausserhalb der Zelle den Stickstoff bindenden Enzyms. Jahrb. wiss. Bot. **83**:567–592. 1936.
8. SHEPHERD, M., An improved apparatus and method for the analysis of gas mixtures by combustion and absorption. Bur. Stand. Jour. Res. **6**:121–167. 1931.
9. VIRTANEN, A. I., Cattle fodder and human nutrition. University Press, Cambridge. 1938.
10. VIRTANEN, A. I., and LAINE, T., N-fixation by excised root nodules. Suomen Kemistilehti **10B**: 24. 1937.
11. ———, Investigations on the root nodule bacteria of leguminous plants. XXII. The excretion products of root nodules. The mechanism of nitrogen fixation. Biochem. Jour. **33**:412–427. 1939.
12. WILSON, P. W., Mechanism of symbiotic nitrogen fixation. Ergeb. Enzymforschung **8**:12–54. 1939.
13. WILSON, P. W., HOPKINS, E. W., and FRED, E. B., The biochemistry of nitrogen fixation by Leguminosae. I. Nitrogen fixation studies of rhizobia apart from the host plant. Archiv. Mikrobiologie **3**:322–340. 1932.
14. WINOGRADSKY, S., Sur le dégagement de l'ammoniac par les nodosités des racines des Légumineuses. Compt. Rend. Acad. Sci. (Paris) **197**: 209–212. 1933.
15. WYSS, O., BURRIS, R. H., and WILSON, P. W., Occurrence and significance of oxalacetic acid in plant tissues. Proc. Soc. Exp. Biol. Med. **40**:372–375. 1939.

MICROSPOROGENESIS AND DEVELOPMENT OF SEED IN LOBELIA CARDINALIS[1]

GEORGE OLDS COOPER

(WITH FORTY FIGURES)

Introduction

Lobelia cardinalis L., a red-flowered member of the Lobeliaceae, grows on low ground and in swampy places. Little, if any, detailed investigation has been done on microsporogenesis and development of the seed in this species. The haploid chromosome number has been reported as seven by DE VILMORIN and SIMONET (10). Early workers on this genus described the development of the ovule through meiosis and, in a few instances, the development of the megagametophyte. The findings of BALICKA-IWANOWSKA (1), BILLINGS (2), and KAUSIK (9) on the development of the megagametophyte agree in general. Their observations on the behavior of certain endosperm cells functioning as haustoria have proved interesting in the light of this present investigation.

The relationship of the vascular strand which supplies the ovule to the development of the endosperm and the embryo has been traced in *L. cardinalis*. This extends the type of observations made by D. C. COOPER (4) on *Phryma leptostachya*, by G. O. COOPER (7) on *Plantago lanceolata*, and by FARLEY and HUTCHINSON (8) on *Medicago*.

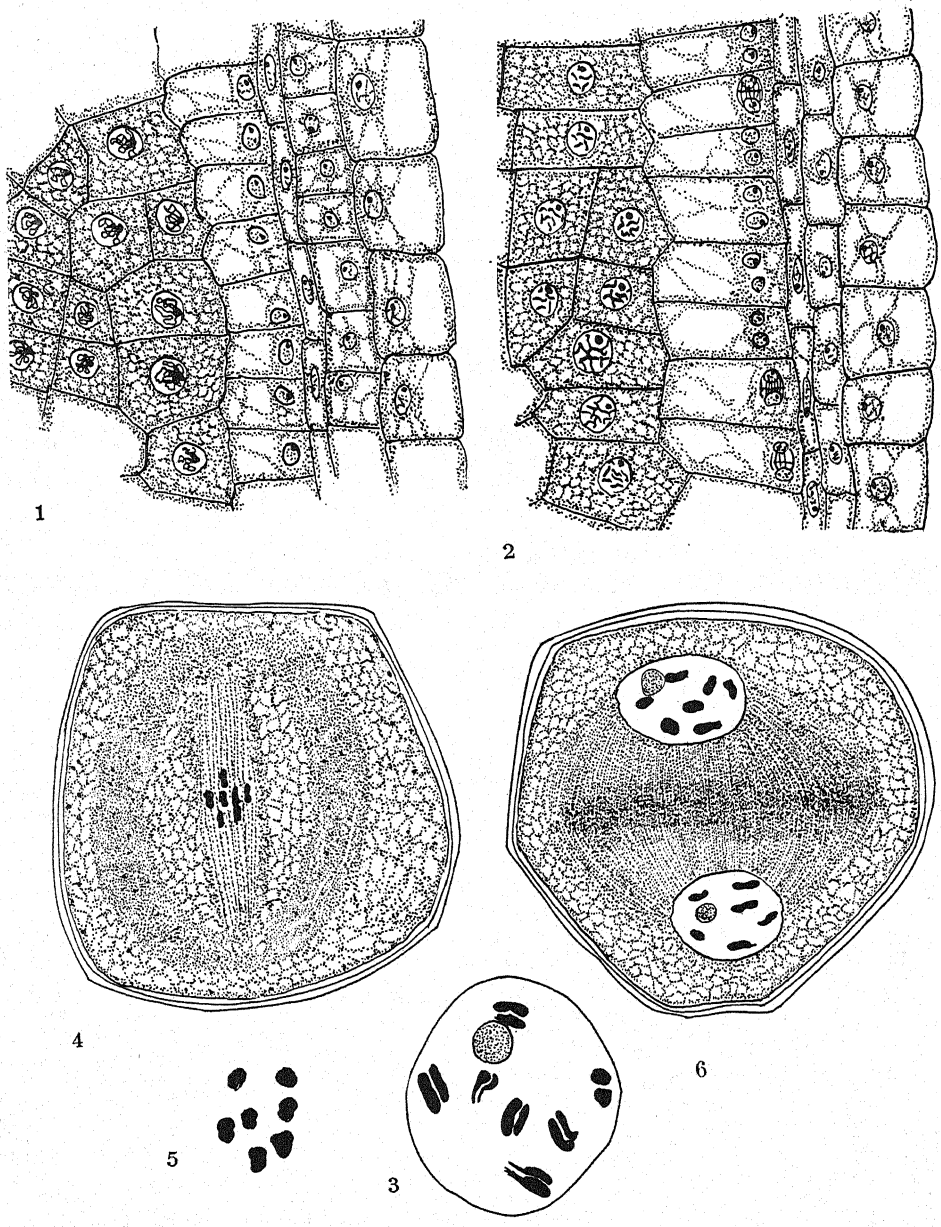
Buds of various stages, open flowers, and young fruits were gathered in late July from plants in the horticultural gardens at the University of Wisconsin. They were fixed either in Carnoy's solution or in Karpenchenko's modification of Navashin's solution, imbedded in paraffin, sectioned at 14 μ, and stained in Delafield's haematoxylin and counterstained with safranin.

Investigation

MICROSPOROGENESIS

Each locule of the anther contains many microspore mother cells, which in turn are surrounded by a conspicuous tapetum. The tapetal cells are uninucleate (fig. 1), later becoming binucleate as the sporogenous cells undergo the prophase stages of meiosis (fig. 2). The nuclei of the microspore mother cells become greatly enlarged during the late prophase stage. Seven pairs of chromosomes are present at diakinesis (fig. 3). At the first meiotic metaphase the nuclear membrane and the nucleolus have disappeared and the chromosomes are massed near the center of the nuclear cavity. A bipolar spindle forms, with the chromosomes arranged on the equatorial plate midway between the poles (fig. 4). Again seven pairs of chromosomes are seen in polar view of the spindle (fig. 5). The cytoplasmic sheath observed by D. C. COOPER (3) and G. O. COOPER (5, 6) is present in this species also. This sheath is more constant than has hitherto been described. The nuclei at interkinesis do not take on the characteristics of resting nuclei and remain for a short time at the

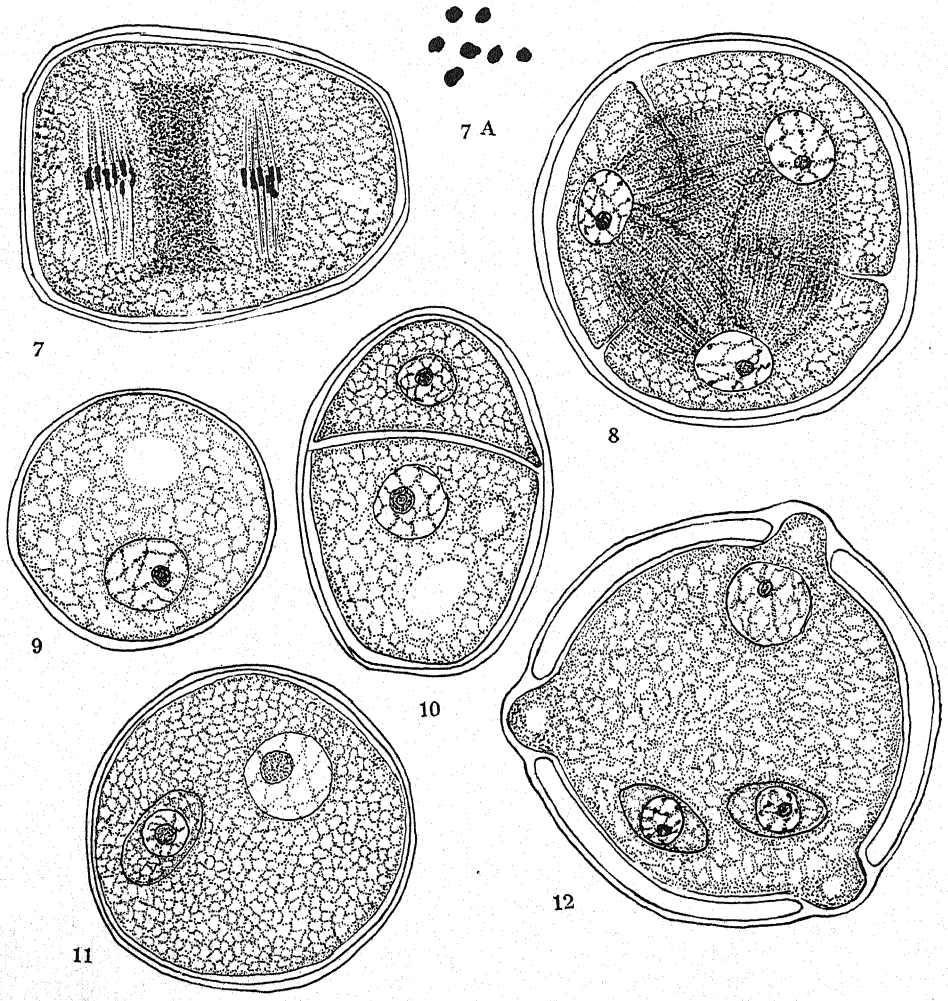
[1] The writer expresses his appreciation to the Departments of Botany and Genetics, University of Wisconsin, for the facilities made available during the course of this investigation.



FIGS. 1–6.—Microsporogenesis: Fig. 1, longisection through anther, showing uninucleate tapetum, nuclei of sporogenous tissue at synizesis; ×700. Fig. 2, tapetal cells binucleate, nuclei of sporogenous tissue at prophase; ×700. Fig. 3, diakinesis, seven pairs of chromosomes; ×3200. Fig. 4, first meiotic metaphase; ×2400. Fig. 5, polar view; ×3200. Fig. 6, interkinesis; ×2400.

late prophase stage (fig. 6). An ephemeral cell plate is laid down and portions of it can be traced into the second meiotic division.

seen in polar view (fig. 7*A*). The second meiotic division results in a cell containing four nuclei. The spindles of this division persist for a time, and cell plates are



FIGS. 7–12.—Microsporogenesis: Fig. 7, second meiotic metaphase; ×2400. Fig. 7*A*, polar view; ×3200. Fig. 8, cytokinesis, partition walls appearing; ×2400. Fig. 9, mature microspore; ×2400. Figs. 10, 11, young pollen grain; ×2400. Fig. 12, mature pollen grain; ×2400.

The spindles of the second meiotic metaphase lie parallel to or at right angles with each other. The ephemeral cell plate of the first meiotic division separates the two spindles (fig. 7). The haploid complement of seven chromosomes is formed midway between the daughter nuclei (fig. 8). Later the cell plates split and four spores are formed, each with a single nucleus. The spores are soon released from the microspore mother cell wall and lie free in the theca. The microspore,

which is slightly ovoid, increases in size, and the wall develops a smooth exine (fig. 9). The nucleus of the microspore migrates to one side of the cell, and the cell divides mitotically to form a large tube cell and a smaller generative cell (fig. 10). The young microgametophyte enlarges further (fig. 11), and shortly before pollination the generative cell divides to form two male gamete cells. The tube cell nucleus at the time of pollination shows signs of disintegration. Three germ pores are arranged equidistant about the pollen grain. A conspicuous vacuole is present within each pore, covered with a thin intine (fig. 12).

Development of ovule

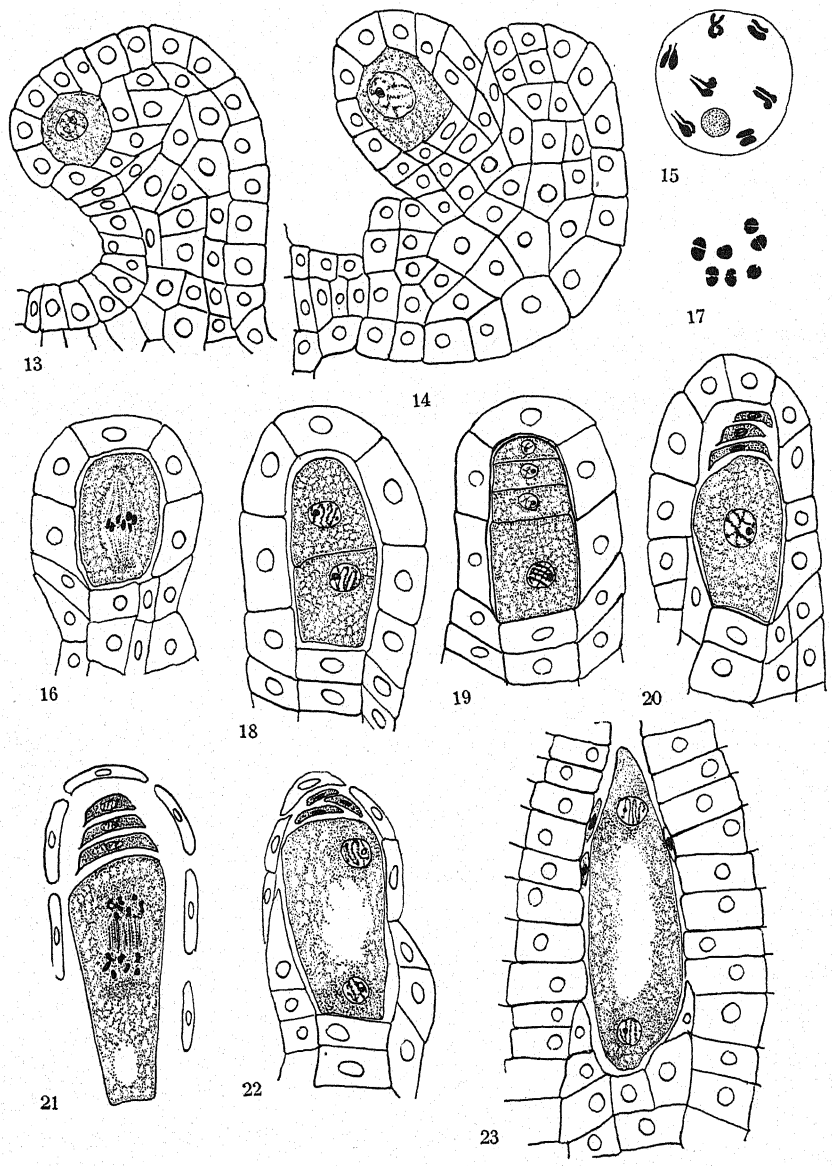
Each flower bears a single pistil composed of two united carpels. Each carpel produces many ovules that are borne attached to the axile placenta. The dividing septum in the course of growth becomes greatly enlarged, is spongy in texture, and is permeated by a much branched vascular system which leads to the many ovules. The ovules appear as rounded outgrowths and arch shortly toward the apex of the ovary. An apical hypodermal cell that functions as the archesporial cell differentiates early (fig. 13). The archesporial cell functions as the megaspore mother cell. As the megaspore mother cell increases in size, a single integument arises as a meristematic outgrowth of the ovule about the nucellus (fig. 14).

Continued growth of the integument and elongation of the funiculus bend the ovule inward, so that the integument almost touches the placenta. This bending is brought about by the more rapid divisions and elongations of those cells in the chalazal region of the ovule opposite the region of the megaspore mother cell, causing the ovule to curve inward more sharply. The continued growth of the integument completely incloses the nucellus, except for a short passage—the micropyle.

When the ovule is partly mature the xylem of the vascular strand can be traced part way into the base of the funiculus. Long, narrow conducting cells extend from the tip of the vascular strand in the funiculus toward the chalazal end of the megagametophyte. Their dense cytoplasmic content and ovoid nuclei distinguish them from the other parenchymatous cells of the ovule (fig. 38). A group of nutritive cells having dense cytoplasmic content are found about the nucellus at the chalazal end of the mature megagametophyte. Small isodiametric cells, which also function as conducting cells, lie between these nutritive cells and the long conducting ones (fig. 39). In the early development of the ovule it is difficult to distinguish these cells from the adjacent ones of the ovule. The parenchymatous conducting cells remain finely vacuolate until the ovule is fairly mature and probably function in osmotic conduction from the vascular strand to the developing seed (fig. 40).

Megasporogenesis

When the integument is forming the megaspore nucleus is approaching synizesis, and at diakinesis seven pairs of chromosomes are present (fig. 15). Following diakinesis, with the disappearance of the nuclear membrane and the nucleolus, seven pairs of chromosomes are arranged on the spindle at the meiotic metaphase (fig. 16). Likewise in polar view the paired nature of the chromosomes can be observed (fig. 17). Two cells are formed as a result of this division (fig. 18). The second meiotic division results in four cells, the larger toward the chalazal end of the ovule becoming the functional



FIGS. 13–23.—Megasporogenesis: Fig. 13, young ovule with hypodermal archesporial cell; ×800. Fig. 14 early development of single integument; ×800. Fig. 15, diakinesis, seven pairs of chromosomes; ×2132. Fig. 16, first meiotic metaphase; ×1000. Fig. 17, polar view; ×2132. Fig. 18, interkinesis; ×1000. Fig. 19, linear row of megaspores; ×1000. Fig. 20, enlarged chalazal megaspore, three disintegrating spores; ×1000. Fig. 21, first division of megaspore nucleus; ×1600. Figs. 22, 23, two-nucleate megagametophyte; fig. 23, nucellar cells toward micropylar end broken down; ×1000.

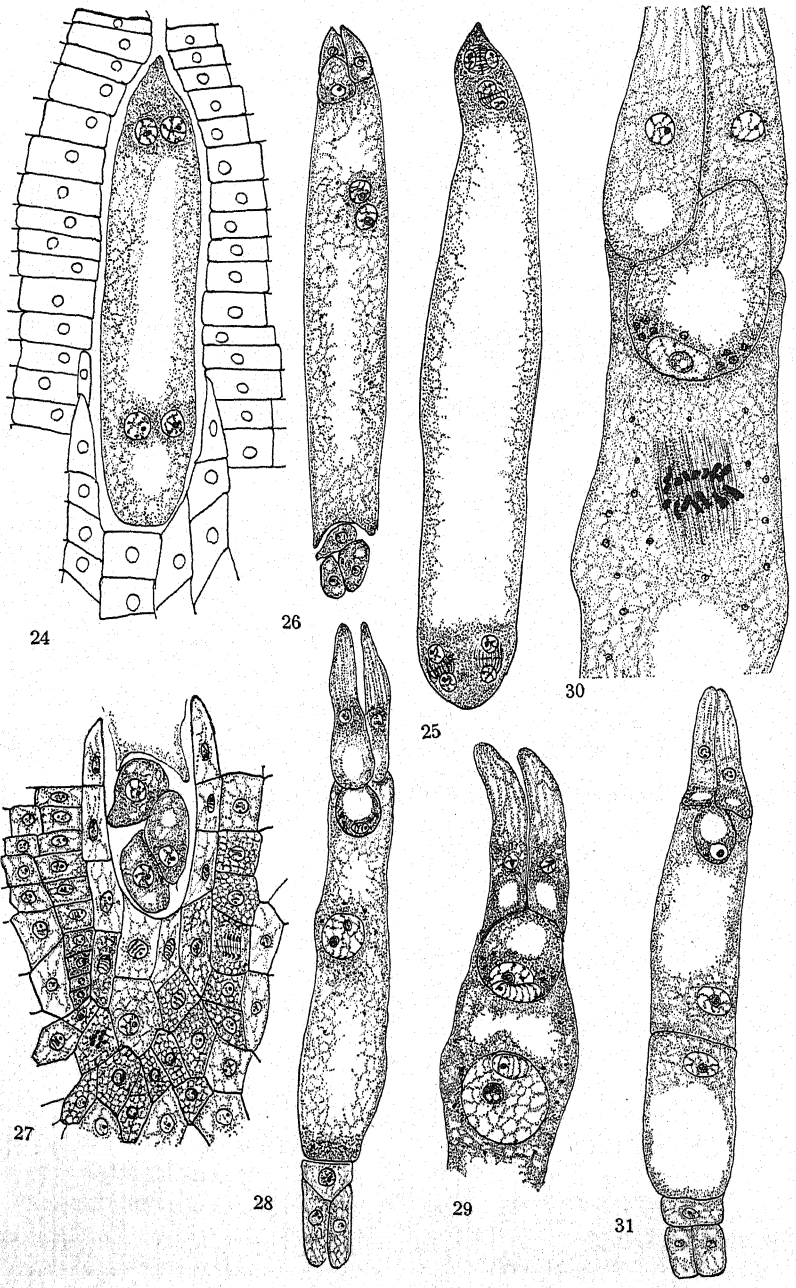
megaspore (fig. 19). The three spores toward the micropylar end soon show signs of disintegration (fig. 20), and upon division of the nucleus of the functional megaspore the nucellar cells in this region break down (fig. 21). The two nuclei from the division of the megaspore nucleus migrate to opposite ends of the cell (fig. 22). Before these nuclei divide again the cell becomes greatly elongated and a large vacuole occupies the mid-portion (fig. 23). These daughter nuclei divide to form a four-nucleate cell (fig. 24), and one further division occurs, producing an eight-nucleate cell (fig. 25). Cell plates are present at this division and ultimately a seven-celled eight-nucleate megagametophyte results (fig. 26). The newly formed megagametophyte consists of three loosely arranged antipodals, a two-nucleate endosperm cell, two linear synergids, and an ovoid egg cell. Starch is present in the egg cell and the endosperm cell. The cells of the endothelial layer of the integument, which now lie adjacent to the megagametophyte, also contain reserve food. The nucellus remains in contact with the megagametophyte only at the antipodal end and is in turn surrounded by a cap of nutritive tissue (fig. 27).

The mature megagametophyte shows a definite arrangement of the antipodals, two of which lie side by side and are adjacent to the nucellus; the third, remaining antipodal, is in contact with the primary endosperm mother cell. Prior to fertilization the two endosperm nuclei fuse and the egg becomes more ovoid, elongating slightly toward the region of the micropyle, and a conspicuous vacuole occupies the center of the cell. The synergids also elongate, the basal vacuoles become more prominent, and the cytoplasm at the apical ends of the cells is more filiform in appearance (fig. 28).

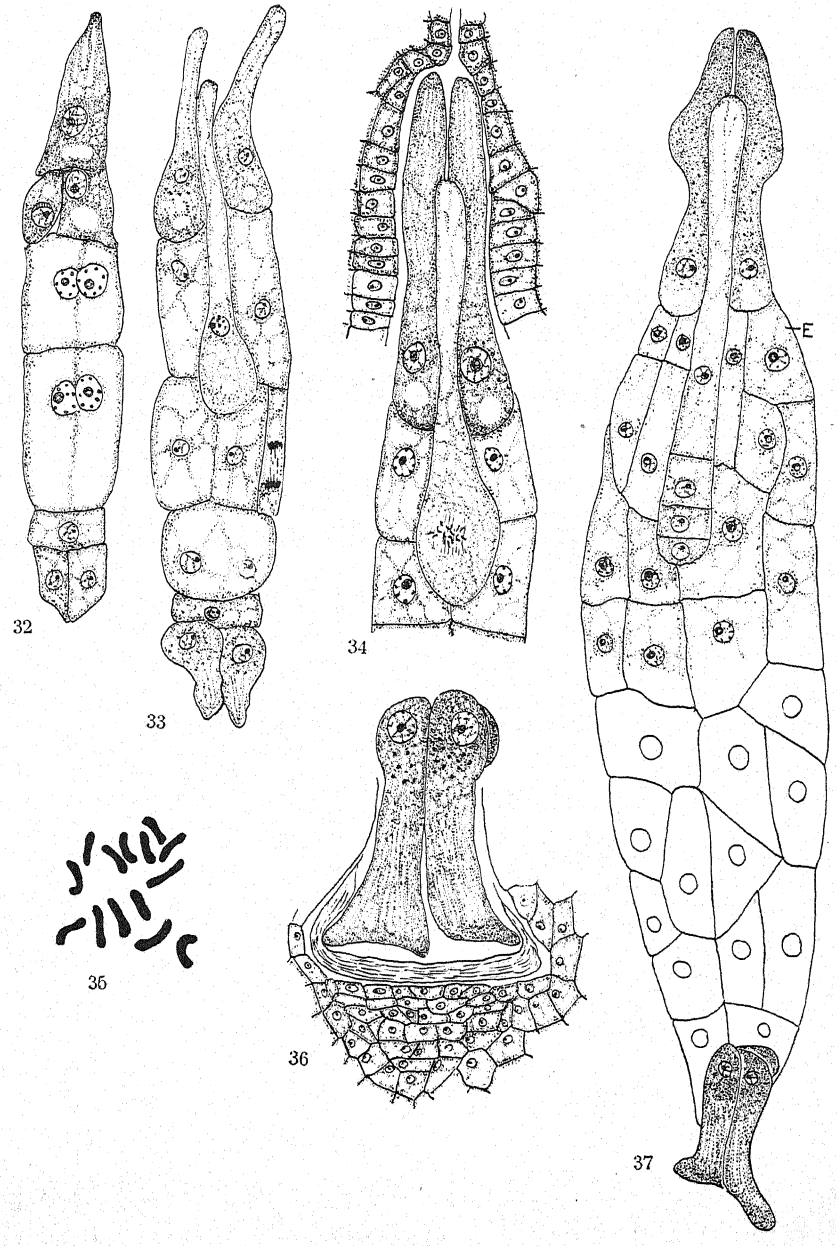
Fertilization and development of endosperm

Several days elapse between pollination and fertilization. One male gamete nucleus unites with the nucleus of the egg; the other becomes associated with the fused nuclei of the endosperm cell (fig. 29). In the primary endosperm cell, which increases in size, the endosperm nucleus and the male gamete nucleus divide simultaneously; at metaphase the chromosomes of the endosperm nucleus and those of the male gamete nucleus move independently toward the midplane of the spindle. The seven chromosomes of the male gamete nucleus are easily distinguished up to metaphase, but the fourteen chromosomes from the endosperm nucleus are not so easily determined here (fig. 30). A two-celled endosperm is now formed, followed by a second division resulting in a quadrant of four cells (figs. 31, 32). The antipodals and synergids persist, become much enlarged, and function as haustoria. The cubical antipodal cell adjacent to the endosperm becomes appressed, owing to growth of the endosperm cells, and comes to lie by the side of the paired antipodals (fig. 36). The cytoplasm in the apical region of these cells has become more vacuolate. The synergids show marked elongation toward the region of the micropyle.

The base of the elongating zygote extends almost to the tips of the synergids and lies between them (fig. 33). The nucleus of the zygote remains in the rounded base of the cell, which divides to form an apical and a long basal cell (fig. 34). Fourteen chromosomes are counted in a polar view of the dividing zygote nucleus (fig. 35). At this time the tips of the two linear antipodals become proliferated, grow as haustoria, and digest the cells of



FIGS. 24–31.—Megasporogenesis and development of endosperm: Figs. 24, 25, four-, eight-nucleate megagametophytes; ×1000. Fig. 26, mature megagametophyte, two polar nuclei; ×466. Fig. 27, nutritive and conducting cells in chalazal region about antipodals and remains of nucellus; ×1000. Fig. 28, mature megagametophyte, fused polar nuclei; ×466. Fig. 29, apex of megagametophyte at time of fertilization; ×800. Fig. 30, division of endosperm nucleus following fertilization; conspicuous chromosomes from male gamete nucleus and smaller chromosomes from fused polar nuclei moving toward mid-plane of spindle; ×1600. Fig. 31, two-cell endosperm; ×466.



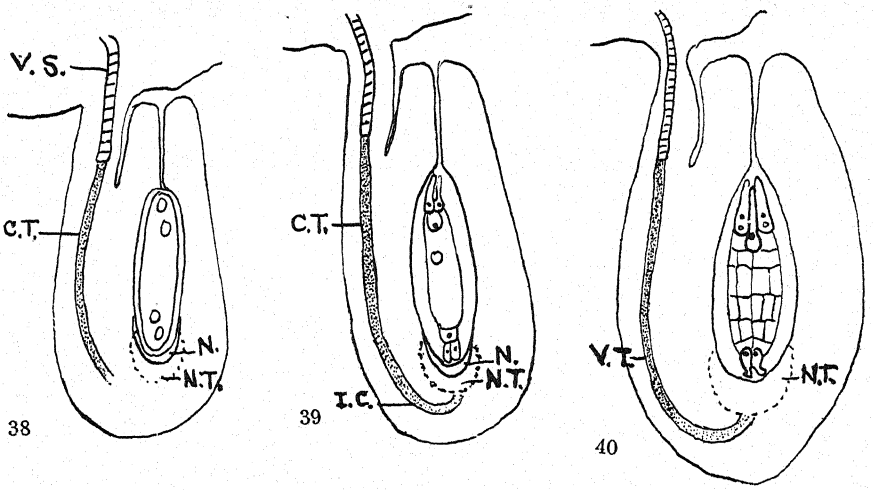
FIGS. 32–37.—Endosperm and development of seed: Fig. 32, four-celled endosperm, enlarging antipodals and synergids; ×466. Fig. 33, further development of endosperm, enlargement of zygote, further increase in size of antipodals and synergids; ×466. Fig. 34, marked enlargement of synergids, zygote at metaphase; ×800. Fig. 35, polar view of zygote nucleus at metaphase, with fourteen chromosomes; ×2166. Fig. 36, enlarged haustorium-like antipodals, collapsed remains of nucellar tissue; ×800. Fig. 37, four-celled embryo surrounded by endosperm tissue (*E*), still functioning haustorium-like synergids, and haustorium-like antipodals; haustorium-like synergids expanding against integument; ×326.

the nucellus so that they become crushed against the nutritive tissue at the chalazal end of the megagametophyte (fig. 36). Later these haustorium-like antipodals digest much of the nutritive tissue. The synergids continue to elongate in the micropyle, and, functioning as haustoria, they digest the adjacent cells of the integument. In the course of their growth the tips of the synergids expand in the cavity left by the digested cells of the mother cells. The tapetal cells are first uninucleate, later becoming binucleate.

2. Cell plates are formed on the cytoplasmic strands midway between the nuclei of the tetrad.

3. The generative cell divides to form two male gamete cells before the pollen grain leaves the anther.

4. Each carpel of the two-loculed pistil contains many ovules, which are anatropous at maturity.



FIGS. 38–40.—Relationship of vascular strand and conductive tissue to development of ovule. Fig. 38, young ovule containing four-nucleate megagametophyte. Vascular strand (*vs*) extends to mid-region of funiculus. Group of elongated cells (*ct*) extend from apex of vascular tissue toward chalaza. *n*, nucellus; *nt*, nutritive tissue. Fig. 39, ovule containing mature megagametophyte. Conducting cells (*ct*) connect vascular strand with isodiametric cells (*ic*) at base of nutritive tissue (*nt*). Fig. 40, early development of young seed. Nucellus completely disappeared and antipodals in direct contact with nutritive tissue (*nt*) at apex of vascular tissue (*vt*).

integument, thereby increasing their absorptive surface. The young seed contains the four-celled embryo surrounded by a many-celled endosperm, with the haustorium-like antipodals digesting the nutritive tissue and the haustorium-like synergids digesting the cells of the integument (fig. 37).

Summary

1. Each theca of the anther of *Lobelia cardinalis* contains many microspore

5. Prior to the formation of the single integument, a single archesporial cell is found in the hypodermal layer of the ovule and functions as the megaspore mother cell.

6. Seven pairs of chromosomes are present at meiosis.

7. A linear row of four megaspores is formed, and the larger spore toward the chalazal end of the ovule is the functional megaspore which develops into an eight-nucleate seven-celled megagametophyte;

the other megaspores disintegrate. The cells of the nucellus toward the micropylar end of the ovule disintegrate upon division of the nucleus of the megaspore.

8. A vascular strand is differentiated early in the development of the ovule and extends part way into the base of the funiculus. Conductive tissue extends from the vascular strand to the nutritive tissue at the chalazal end of the megagametophyte.

9. Double fertilization occurs. At metaphase in the first division of the endosperm nucleus the chromosomes of the endosperm nucleus and those of the male gamete nucleus move independently toward the mid-plane of the spindle. A four-celled endosperm is formed prior to division of the zygote nucleus.

10. The synergids and the antipodals function as chalazal and micropylar haustoria, respectively. The synergids become greatly enlarged and digest the cells of the integument about the micropyle. The nucellar cells at the chalazal end of the megagametophyte are digested by the antipodals, which later digest the cells of the adjoining nutritive tissue.

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LITERATURE CITED

1. BALICKA-IWANOWSKA, G., Contribution à l'étude du sac embryonnaire chez certaines Gamopetales. Flora **86**:47–71. 1899.
2. BILLINGS, F. H., Beitrage zur Kenntnis der Samenentwicklung. Flora **88**:253–318. 1901.
3. COOPER, D. C., Microsporogenesis and the development of the male gamete in *Portulaca oleracea*. Amer. Jour. Bot. **22**:453–459. 1935.
4. ———, Macrosporogenesis and the development of the seed of *Phryma leptostachya*. Amer. Jour. Bot. **28**:755–761. 1941.
5. COOPER, G. O., Cytological studies in the Chenopodiaceae. I. Microsporogenesis and pollen development. BOT. GAZ. **97**:169–178. 1935.
6. ———, Cytological investigations of *Pisum sativum*. BOT. GAZ. **99**:584–591. 1938.
7. ———, Development of the ovule and the formation of the seed in *Plantago lanceolata*. Amer. Jour. Bot. **29**: 577–581. 1942.
8. FARLEY, H. M., and HUTCHINSON, A. H., Seed development in *Medicago* (alfalfa) hybrids. I. The normal ovule. Canad. Jour. Res. **19**:421–437. 1941.
9. KAUSIK, S. B., Gametogenesis and embryogeny in *Lobelia nicotianaefolia* Heyne. Jour. Ind. Bot. Soc. **17**:161–168. 1938.
10. VILMORIN, R. DE, and SIMONET, M., Nombre des chromosomes dans les genres *Lobelia, Linum* et chez quelques autres espèces végétales. Compt. Rend. Soc. Biol. Paris **96**:166–168. 1927.

VOLATILE SULPHUR CONTENT OF BLACK MUSTARD PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 544

SCOTT V. EATON

Introduction

In connection with a study now in progress of the effects of sulphur deficiency on the metabolism of the black mustard plant, *Brassica nigra* (L.) Koch., it became necessary to determine the volatile sulphur content of the fresh plant tissue. This necessity arose from the fact that total sulphur was determined in tissue dried in the vacuum oven. During the drying much of the volatile sulphur would escape from the tissue. A survey of the literature showed that, while allyl mustard oil (the volatile sulphur compound of the seed) has been much investigated in seed tissue, there has been little work on the plant itself. After the method had been mastered, therefore, it was decided to study the distribution of volatile sulphur in the plant and the effect of various conditions on the amount present. It seems advisable to report these data in a separate paper.

Methods

Considerable preliminary work was done on methods of determining volatile sulphur. In connection with a study of the effects of sulphur deficiency on the metabolism of the soybean (2), PETERSON'S (11) method was tried. It did not seem to give very good results. In a similar study of the sunflower (3), the method of PLATENIUS (8) was used. This method seemed satisfactory, but it was not fully investigated at the time because the tests indicated that little if any volatile sulphur was present. It was thought that there would be no error in determining total sulphur in the dried residue.

The method of PLATENIUS was developed for determining the pungency of onions. It was realized that the volatile sulphur compounds of the onion and of the black mustard plant are different, and that therefore the method might have to be modified somewhat. Considerable preliminary work was done to determine the proper time of hydrolysis, temperature of distillation, and amount of distillate necessary to free the tissue of volatile sulphur. The tests indicated that the method as outlined by PLATENIUS would be satisfactory in the case of the mustard plant, and it was therefore used without any essential modification.

The method is one of steam distillation. Briefly it consists of heating the tissue in dilute acid at a temperature of 122°–124° C. to hydrolyze the glucoside. Then salt is added to increase the boiling point and the distillation is carried on at a higher temperature, first at 135° and finally at 145° C., until 800 ml. of distillate has been collected. The distillate is collected in saturated bromine water. The bromine oxidizes the sulphur to sulphate, which is determined as barium sulphate.

The size of the sample of the present work, usually 100 gm., was considerably less than the samples used by PLATENIUS, so the difference in the amount of tissue water added was made up with distilled water in order to keep the concentration of acid the same. Preliminary work indi-

cated that, if during distillation the acid became too concentrated, erratic results would be obtained. Allyl mustard oil boils at 150.7° C. It might seem necessary to use a temperature at least as high as this for the distillation. But in tests, as much sulphur was obtained by distillation at 145° as at 155° C. In fact, when the entire distillation was carried on at 122°–124° C., as much sulphur was obtained as at 145° or 155° C. A much longer time was of course required at the lower temperature to obtain the 800 ml. of distillate. The volatile sulphur would pass off rather rapidly at temperatures below its boiling point and would also tend to be carried over mechanically by the steam.

The plants were grown in the greenhouse during the period from February 22 to July 6, 1941. The seed was obtained from Sutton and Sons, England. There were a number of crops. The number of plants of the various crops varied from 140 to 300 or more. The growth period of each crop is given in table 1. In order that the plants of all the crops might be of the same physiological age at the time of harvest, the samples were taken when the first flower buds were observed on any given crop. Because of photoperiodic effects, and partly owing to temperature differences, the length of the growth period of the crops varied considerably.

Data

Table 1 gives the data for the percentage water, percentage dry matter, and percentage volatile sulphur estimated both as sulphur and as allyl mustard oil. Since the percentage dry matter varies decidedly in several cases, the data are given on both a green- and a dry-weight basis. In the table are given the results of each individual analysis. Except the data for the leaves and stems of the plants grown in sand, each pair of determinations (only one determination was made of roots) does not represent duplicates. The plan of making the analyses was to take from the same crop on a given day two samples, one for each condition or part of plant being compared; then a little later two other samples of this same crop were taken. Thus, except in the case of the roots, two determinations on each plant part and for each condition were made but on different days and therefore on plants of slightly different age. This method of analysis was necessary since it was not possible to attend to more than two pieces of apparatus at the same time. Only the blades of the leaves were analyzed; the petioles were not included.

As shown by the table, the leaves of plants grown in sand contain more volatile sulphur than the leaves of plants grown in soil. As to the distribution in the plant, the leaves contain considerably more volatile sulphur than the stems. The roots of only one of the sand cultures were analyzed. Their pungency is intermediate between leaves and stems estimated on a green-weight basis but less than either of the other parts on a dry-weight basis. The upper leaves of the plant are considerably more pungent than the lower. Growing the plants in a minus-sulphur nutrient solution results in almost complete lack of development of volatile sulphur. On the other hand, minus-nitrogen leaves are slightly more pungent than plus-nitrogen ones. Considering the effect of the age of the plants, the leaves of younger plants contain more volatile sulphur than those of older ones. There is not a regular decrease in pungency as the age of the plant increases, however, though in the case of the three sand cultures the decrease is regular. The percentages for each age are averages of data

TABLE 1

PERCENTAGE WATER, DRY MATTER, AND VOLATILE SULPHUR

GROWTH PERIOD	PERCENTAGE		VOLATILE SULPHUR (CALCULATED AS S)		VOLATILE SULPHUR (CALCULATED AS ALLYL MUSTARD OIL)	
			PERCENTAGE			
	WATER	DRY MATTER	GREEN WEIGHT	DRY WEIGHT	GREEN WEIGHT	DRY WEIGHT
	PLANTS GROWN IN SOIL Leaves					
2/22 to 4/15 (53 days)	89.430	10.570	0.0080	0.0760	0.0247	0.2351
2/22 to 4/18 (56 days)	88.997	11.003	0.0084	0.0768	0.0260	0.2374
	Stems					
2/22 to 4/15 (53 days)	93.318	6.682	0.0042	0.0627	0.0130	0.1938
2/22 to 4/18 (56 days)	92.971	7.029	0.0034	0.0479	0.0105	0.1480
	PLANTS GROWN IN SAND Leaves					
6/3 to 7/4 (31 days)	89.524	10.476	0.0116	0.1108	0.0359	0.3426
6/3 to 7/4 (31 days)	89.458	10.542	0.0122	0.1152	0.0377	0.3563
	Stems					
6/3 to 7/6 (33 days)	92.530	7.470	0.0056	0.0746	0.0173	0.2306
6/3 to 7/6 (33 days)	93.640	6.360	0.0060	0.0948	0.0186	0.2931
	Roots					
6/3 to 7/5 (32 days)	82.772	17.228	0.0112	0.0704	0.0346	0.2177
	POSITION OF LEAVES ON PLANT Leaves of upper half					
3/23 to 5/2 (40 days)	88.618	11.382	0.0100	0.0887	0.0309	0.2742
3/23 to 5/5 (43 days)	86.705	13.295	0.0155	0.1162	0.0479	0.3594

TABLE 1—*Continued*

Growth period	Percentage		Volatile sulphur (calculated as S)		Volatile sulphur (calculated as allyl mustard oil)	
			Percentage			
	Water	Dry matter	Green weight	Dry weight	Green weight	Dry weight
Leaves of lower half						
3/23 to 5/2 (40 days)	91.927	8.073	0.0017	0.0213	0.0053	0.0657
3/23 to 5/5 (43 days)	90.472	9.528	0.0021	0.0224	0.0065	0.0692
Effects of sulphur deficiency Minus-sulphur leaves						
4/5 to 5/17 (42 days)	88.925	11.075	0.0003	0.0031	0.0009	0.0096
4/5 to 5/21 (46 days)	89.638	10.362	0.0013	0.0126	0.0040	0.0389
Plus-sulphur leaves						
4/5 to 5/17 (42 days)	88.718	11.282	0.0091	0.0806	0.0281	0.2503
4/5 to 5/21 (46 days)	89.513	10.487	0.0102	0.0976	0.0315	0.3017
Effects of nitrogen deficiency Minus-nitrogen leaves						
4/19 to 5/24 (35 days)	85.229	14.771	0.0130	0.0882	0.0402	0.2726
4/19 to 5/25 (36 days)	84.867	15.133	0.0150	0.0989	0.0464	0.3057
Plus-nitrogen leaves						
4/19 to 5/24 (35 days)	88.768	11.232	0.0100	0.0886	0.0308	0.2741
4/19 to 5/25 (36 days)	88.304	11.696	0.0100	0.0851	0.0308	0.2633
Age of plant Leaves						
31 days (sand culture)	89.487	10.513	0.0119	0.1130	0.0367	0.3496
35–36 days (sand culture)	88.537	11.463	0.0100	0.0869	0.0308	0.2686
40–43 days (soil culture)	89.789	10.211	0.0080	0.0742	0.0246	0.2294
42–46 days (sand culture)	89.126	10.874	0.0097	0.0890	0.0299	0.2751
53–56 days (soil culture)	89.208	10.792	0.0082	0.0764	0.0255	0.2362

given elsewhere in the table. Owing to the variation in amount of dry matter in the samples, the differences in volatile sulphur are considerably less in several cases when calculated on a dry-weight than on a green-weight basis.

Discussion

The pungency of crushed black mustard seed is due, mainly at least, to allyl mustard oil, 2-propenyl isothiocyanate ($CH_2CHCH_2 \cdot NCS$). This essential oil occurs in plants as a component of the glucoside sinigrin. In the seed of the black mustard, the glucoside and the enzyme myrosin that hydrolyzes it are found in different cells, so that the oil is not released until by the crushing of the tissue the enzyme and glucoside are brought together and the glucoside is hydrolyzed (1). This separation of the glycoside and the enzyme that hydrolyzes it seems a rather general situation in plants. In the case of cruciferous species, it is well known that their characteristic sharp odor does not arise until the tissue is crushed. The equation for the hydrolysis of sinigrin is:

$$C_{10}H_{16}O_9NS_2K + H_2O \xrightarrow{\text{Myrosin}} C_3H_5 \cdot NCS + C_6H_{12}O_6 + KHSO_4 .$$

While sinigrin has been studied mainly in the seed of the black mustard, it has been demonstrated in a number of other cruciferous species. These include *Thlaspi arvense* (field pennycress), *Armoracia rusticana* (horseradish), *Alliaria officinalis* (hedge garlic), and *Brassica rapa* (turnip) (6).

Allyl mustard oil is a limpid colorless oil which, as already stated, boils at 150.7° C. It has a sharp odor and taste. If brought into contact with the skin, blisters may be caused. The bactericidal and fungicidal properties of the compound have been demonstrated (5, 15).

The present work was concerned with the plant itself; no work was done on the seed. Although it is known that the essential oils of the different parts of a plant may be different in character (12), it is assumed that allyl mustard oil also accounts for the pungency of the leaves, stems, and roots of the plant. As shown by table 1, the leaves contain much more volatile sulphur than the stems. The roots are intermediate between leaves and stems in volatile sulphur when estimated as percentage of green weight, but the percentage on a dry-weight basis is lower than that of both leaves and stems. The data for the roots, however, should be regarded merely as indicative. The roots of only the sand culture were analyzed and only a single determination made. In regard to the roots, the green-weight data especially should not be overemphasized, for it was impossible completely to dry the roots after washing them from the sand. Each part of the plant is much lower in the oil than the seed. WINTON and WINTON (16) give the data of HUBER and VAN DER WIELEN for the allyl mustard oil content of black mustard seed from different countries. The range is from 0.63 to 1.23 per cent. The largest amount found in the present study for any plant part was 0.3594 per cent in the upper leaves of 43-day plants.

Tissue of high water content is likely to be low in volatile sulphur, at least when calculated on a green-weight basis, for a sample of such tissue contains less dry matter than the same weight of tissue lower in water and is likely to contain less volatile sulphur—one constituent of the dry matter. But this is not necessarily true. Plant material high in water may contain so much volatile sulphur that even its green-weight percentage is greater than tissue relatively low in water. JONES and BISSON (7) concluded

that mild varieties of onions have the highest water content and the most pungent varieties the lowest. PLATENIUS and KNOTT (10) grew a number of varieties of onions in soil and also found that in general the pungency varied inversely with the water content; but the relationship was not exact. For example, the most pungent variety and two decidedly less pungent ones had about the same water content. Irrigated onions contained more water and less volatile sulphur than nonirrigated ones. The former bulbs were larger than the latter, however, and the increased production of tissue might have been an additional factor in decreasing the pungency. There may not have been enough sulphur in the soil to make the larger amount of tissue as high in volatile sulphur as the smaller nonirrigated bulbs. This inverse relationship of pungency and water content appears in the present work in the comparison of the volatile sulphur content of leaves and stems, lower with upper leaves, and plus-nitrogen with minus-nitrogen leaves. In each case the tissue with the higher water content has also the lower fresh-weight percentage of volatile sulphur. The dry-weight percentages show a similar but smaller difference, indicating that the decreased pungency of the tissue of higher water content is not due entirely to a dilution of the volatile sulphur with water, but that there is actually less volatile sulphur in proportion to the remainder of the dry matter.

The age of the leaves affects their volatile sulphur content. This is shown especially in comparing the upper leaves of the plant with the lower. The former are much more pungent. A similar difference but of less magnitude is seen when the leaves of younger plants are compared with those of older ones. The younger the plant, the more volatile sulphur is found in its leaves, although there is not an exactly regular decrease in pungency as the age of the leaves increases. In obtaining these data, all the leaves of the plants were analyzed. It must be kept in mind that the plants of the various ages were grown at different times and that some were grown in soil and some in sand. PLATENIUS and KNOTT (10) have shown that climatic conditions and the amount of sulphur in the soil affect the volatile sulphur content of onions. Three of the groups of plants, those of 31 days, 35–36 days, and 42–46 days, were grown in sand with the same nutrient solution, and in the leaves of these plants there is a regular decrease in pungency as the age of the plants increases. But since the plants were grown at different times, there is a possibility that differences in climatic conditions might account in part for the variations in volatile sulphur.

Sufficient work was not done to give a basis for explaining the greater pungency of younger leaves. Two possibilities may be mentioned. As the leaves become older there may be translocation of the volatile sulphur compounds from the older leaves to other parts of the plant; for example, from the lower to the upper leaves. PLATENIUS and KNOTT (9) found that the percentage of volatile sulphur of onion bulbs increased steadily up to near the time at which the tops began to fall over. If, as seems likely, the volatile sulphur compounds are made mainly in the leaves, there was constant translocation from the leaves. Another possibility is that as the leaves become older the membranes become more permeable. This may result in contact of the glucoside and enzyme and hydrolysis of the glucoside. The resulting volatile sulphur would gradually escape from the plant.

When plants were grown in minus-sul-

phur nutrient solutions the leaves tasted flat, as compared with the sharp acrid taste of plus-sulphur leaves. Analysis showed that the minus-sulphur leaves were almost completely lacking in volatile sulphur. This is perhaps to be expected, since sulphur is a component of allyl mustard oil. Also, when the plants were grown in sand with a complete nutrient solution, their leaves contained more volatile sulphur than when grown in soil. All the sulphur of the nutrient solution was in an available form, while only part of that of the soil was in this state. PLATENIUS and KNOTT (10) grew onions in several types of soil and found a definite though not proportional relationship between pungency and the sulphur content of the soil. They call attention to the demand for both mild and pungent varieties of onions for food, but decide—because of the results of their fertilizer experiments—that it is not feasible to increase the pungency by sulphur fertilizers. The addition of 600 pounds of sulphur per acre to a sandy soil did not increase the volatile sulphur content of the onions significantly. It is possible that the pungency of cruciferous plants could be varied more widely by growing the plants with nutrient solutions of varying sulphur content by one of the soilless methods (14).

There is no indication from this work that the sulphur of the air affects the volatile sulphur content of black mustard leaves. The atmosphere of Chicago contains much sulphur, especially during the winter and early spring (4), and experiments have indicated that sulphur dioxide in low concentrations in the air may be utilized by plants (13). But in general the earlier in the year the plants were grown the less volatile sulphur did their leaves contain. With the exception that the seeds of the 40–43-day plants were planted before those of the 42–46-day plants, the longer the growth period the earlier in the season were the seeds planted. And the leaves of these older plants contained in general less volatile sulphur than did those of the younger plants. It would seem that the age of the plant and perhaps also certain climatic conditions are more important than atmospheric sulphur in affecting the pungency of the leaves.

Nitrogen is also a component of allyl mustard oil. But growing the plants in minus-nitrogen nutrient solutions increased rather than decreased the volatile sulphur content of the leaves. This is in line with the common observation that stunted cruciferous plants are more pungent than larger ones. The minus-nitrogen plants were much smaller than the plus-nitrogen ones, and on the per-plant basis the leaves of the former plants contained much less volatile sulphur than did those of the latter. The sulphur content of the nutrient solutions used in growing the two sets of plants was the same. Since the pungency of cruciferous plants seems to vary directly with the amount of sulphur present in an available form, it is probably to be expected that the small leaves of the minus-nitrogen plants would contain a higher percentage of volatile sulphur than the much larger plus-nitrogen leaves.

Summary

1. The leaves of the black mustard plant contained more volatile sulphur than the stems. The work on the roots was inconclusive.

2. The leaves contained more volatile sulphur when the plants were grown in sand with a complete nutrient solution than when grown in soil.

3. Sulphur and nitrogen are both com-

ponents of allyl mustard oil. Growing the plants in a minus-sulphur nutrient solution caused an almost complete loss of pungency of the leaves. Minus-nitrogen leaves were somewhat more pungent than plus-nitrogen ones.

4. Young leaves contained more volatile sulphur than older ones. The upper leaves of the plant were more pungent than the lower and the leaves of plants grown a short time than those of plants grown a longer time.

5. Tissue of high water content contained less volatile sulphur than tissue of lower water content.

UNIVERSITY OF CHICAGO

LITERATURE CITED

1. ARMSTRONG, E. F., and ARMSTRONG, K. F., The glycosides. New York. 1931.
2. EATON, S. V., Influence of sulphur deficiency on the metabolism of the soybean. BOT. GAZ. 97:68–100. 1935.
3. ———, Influence of sulphur deficiency on metabolism of the sunflower. BOT. GAZ. 102: 536–556. 1941.
4. EATON, S. V., and EATON, J. H., Sulphur in rainwater. Plant Physiol. 1:77–87. 1926.
5. FOTER, M. J., Bactericidal properties of allyl isothiocyanate and related oils. Food Res. 5:147–152. 1940.
6. GILDEMEISTER, E., and HOFFMAN, FR., The volatile oils. Vols. I, II. New York. 1913; 1916.
7. JONES, H. A., and BISSON, C. S., Moisture content of different varieties of onions. Amer. Soc. Hort. Sci. Proc. 31:165–168. 1934.
8. PLATENIUS, HANS, A method of estimating the volatile sulphur content and pungency of onions. Jour. Agr. Res. 51:847–853. 1935.
9. PLATENIUS, HANS, and KNOTT, J. E., The pungency of the onion as influenced by the stage of development of the plant. Amer. Soc. Hort. Sci. Proc. 33:481–483. 1935.
10. PLATENIUS, HANS, and KNOTT, J. E., Factors affecting onion pungency. Jour. Agr. Res. 62: 371–379. 1941.
11. PETERSON, W. H., Forms of sulphur in plant materials and their variations with the soil supply. Jour. Amer. Chem. Soc. 36:1290–1300. 1914.
12. POWER, F. B., The distribution and characters of some of the odorous principles of plants. Jour. Ind. Eng. Chem. 11:344–352. 1919.
13. SETTERSTROM, CARL, ZIMMERMAN, P. W., and CROCKER, WILLIAM, Effect of low concentrations of sulphur dioxide on yield of alfalfa and Cruciferae. Contrib. Boyce Thompson Inst. 9:179–198. 1938.
14. TURNER, W. I., and HENRY, V. M., Growing plants in nutrient solutions. New York. 1939.
15. WALKER, J. C., MORRELL, SAM, and FOSTER, H. H., Toxicity of mustard oils and related sulphur compounds to certain fungi. Amer. Jour. Bot. 24:536–541. 1937.
16. WINTON, A. L., and WINTON, KATE B., The structure and composition of foods. Vol. IV. New York. 1939.

CELL SIZE IN DEVELOPING OVARIES OF IRIS FULVA[1]

HERBERT PARKES RILEY AND DOROTHY MORROW

(WITH SEVEN FIGURES)

It has been shown (2) that, when the ovary of *Iris fulva* is first organized, it has in cross-section six small projections and also three indentations at the septa and three slight identations at the midribs of the carpels. The projections represent ridges which run lengthwise, and the indentations represent furrows that also extend the length of the ovary. As the ovary develops, these ridges and furrows become increasingly prominent until sometime after fertilization; then they become less and less so, until in the mature capsule they are practically obliterated.

The changes in shape which occur during development are the result of differences and changes in the rates of relative growth of the three main radii of the carpels: the midrib, the septum, and the "widest radius"—which approximately bisects the angle formed by the midrib and the septum (2). The placental region of one radius grows at the same relative rate as that of any other radius, while the locular region at one radius grows at essentially the same relative rate as that at any other. The ovary walls, however, frequently grow at very different relative rates, and these rates may change radically at different stages of development. When these radii are divided into their component parts, therefore, differences in ovary shape are seen to be a function, not only of differences in the relative growth rates of these radii themselves but even more specifically of differences in the rates of relative growth of the ovary wall at these radii.

The ovary wall at all radii and the locular region at the septum are cellular structures. The purpose of this investigation was to determine to what extent the differences and changes in the relative growth rates of these regions are due to differences and changes in the size of their cells and to what extent to differences and changes in the rate of cell division. A preliminary report has been published (4).

Some of the earlier work on cell size and cell division in a developing organ has been discussed by SINNOTT (5). In developing fruits, earlier growth is due largely to cell multiplication, while later growth is due to cell expansion, as HOUGHTALING (1) showed for tomatoes. SINNOTT (5) reported essentially the same condition in cucurbit fruits. In the developing ovary he found that early growth is due chiefly to cell multiplication, although cell size also increases during this period. After a specific cell size is reached, cell multiplication ceases and growth of the ovary is due to cell enlargement alone. Huxley's heterogonic growth formula, $y = bx^k$, which can be written $\log y = \log b + k \log x$, is based on the assumption that the relative growth rates of any two components are proportional. From the logarithmic form it is seen that this would yield a straight line when plotted. Letting x equal the width of a component of the radius and y the width of the cells making up this component yields a polygonal

[1] This study was aided by an award from the New Orleans Academy of Science.

curve rather than a single straight line. The bends in the curve may be attributed to the radical changes in the rates of growth at the different stages of development. The polygonal curve takes the form of a straight line whose slope is less than one; there is then a decided break in the curve and the remainder of it is a straight line whose slope equals one. SINNOTT points out that during the early stages there is considerable increase in cell diameter, even while cell division is going on, and therefore there is no fixed size at which cell division occurs. Division of cells stops suddenly rather than gradually, showing that cell division ceases when a definite cell size is attained. The curve of relative growth follows exactly the same pattern for the twelve races of cucurbits examined, although the exact slopes of the line are not the same in all cases.

Material and methods

The plants used for this study were from a region several miles north of Thibodaux, Louisiana. The material was collected, fixed, and imbedded weekly during the spring of 1938, while the senior writer was at Tulane University. Subsequent sectioning, staining, and drawing and measuring of the cells were carried out at the University of Washington. This paper, therefore, represents a joint contribution from the departments of botany of both institutions.

The slides from which the measurements were taken are the same as those used by the senior writer for an analysis of relative growth of the entire radii and their component parts (2). The relative growth curves of ovary wall at the midrib and ovary wall at the widest radius, of ovary wall at the midrib and ovary wall at the septum, and of ovary wall at the septum and locular region of the septum (2) will be referred to in this paper.

Measurements of cell size were obtained from camera lucida drawings of parenchyma cells by means of a scale drawn from a stage micrometer. Each measurement is the average of thirty-five cells. Only parenchyma cells were measured, although a vascular bundle is present in the wall at both the midrib and the septum. Each measurement represents, not the average diameter of the cell but the diameter of the cell measured in the direction parallel to the radius in which it lies. As the parenchyma cells of the wall are approximately isodiametric in all but the final stage of development of the capsule, the measurements are approximately the average diameters of the cells in the earlier stages. In the final stages of development, the cells tend to elongate so that their longest diameter is perpendicular to the radius or at an angle of less than 90° to it; these measurements are therefore across or at an angle to the long dimension of the cells. Measurements of cell size can be considered reliable for the earlier stages, as the amount of variation was slight in all cases; but in the last stages of the capsule the measurements tend to be less accurate, since there is more variation in cell size and in the angle of the cell to the radius, and since a number of cells appear to be distorted as the result of stretching caused by expansion of the locules. The cells in the locular region of the septum are generally elongated in the plane of the radius, so measurements of these cells represent measurements of cell length.

Measurements of cell size were plotted logarithmically against the ovary wall or locular region, and measurements of cell size in one region were plotted against those in another region. The relative

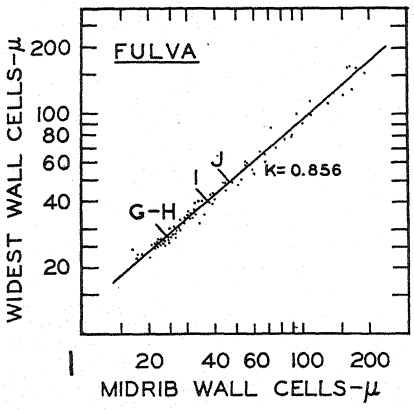
growth rates, k, were obtained from Huxley's formula. Except for the very short parts of some curves, they were plotted by the method of least squares. Corresponding parts on the several curves are identified by the letters G, H, I, or J, the last three letters corresponding to similar places in the curves discussed previously (2). Point G is just before and point H just after meiosis of the megasporocyte; point I is just at or just slightly after the beginning of fertilization; point J is very shortly after fertilization and just as the seed is beginning to enlarge. The various events in the development of the megaspores and megagametophyte are described elsewhere (3).

The values of k have been calculated to three decimal places, although it is doubtful whether there is much significance in the third place, and probably even the second place is doubtful for many sections of the curves, as the points frequently show some scatter. This is especially true toward the end of development, since the mature capsules (and especially their cells) show more variability than do ovaries and young capsules. The calculation of standard errors for k values seemed unnecessary, for the main interest is in the general trends of the curves.

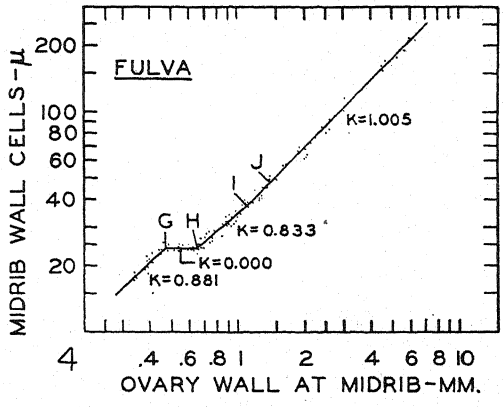
Observations

The ovary wall at the widest radius grows somewhat more rapidly than the wall at the midrib before fertilization, much more rapidly during fertilization, and somewhat more slowly thereafter. As figure 15 of the previous paper (2) shows, $k = 1.343$ until just before fertilization, 1.985 from then until just after fertilization, and 0.839 for the remainder of ovary development. During the entire development of the ovary, the cells of the wall at the midrib grow somewhat more rapidly than do those at the widest radius, and there is no change in the relative growth rate (fig. 1). The ovary wall at the midrib grows slightly more rapidly than the wall at the septum ($k = 0.943$) before fertilization, but the wall at the septum grows much more rapidly during fertilization ($k = 2.803$) and very slightly faster ($k = 1.071$) thereafter (2, fig. 19). The cells of the wall at the midrib and septum grow at almost the same relative rate, and this rate remains the same throughout development (fig. 2). The relationship of the ovary wall at the septum and the locular region of the septum is interesting (2, fig. 21). The locular region grows much more rapidly until just after meiosis ($k = 4.155$), at almost the same rate from that time until just before fertilization ($k = 1.053$), then much more slowly during fertilization ($k = 0.401$), and somewhat more rapidly for the remainder of capsule development ($k = 1.449$). The cells of the wall increase in size somewhat more rapidly than do those of the locular region (fig. 3) throughout development, and there are no changes in the slope of the line for the entire curve.

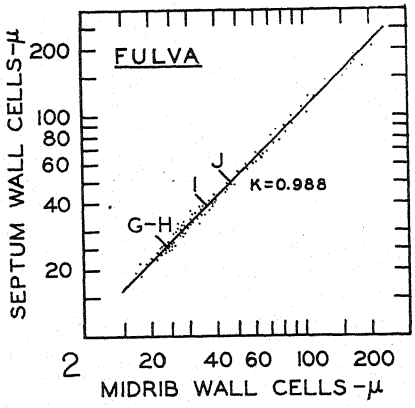
The complexity of the curves when one region is plotted against another (2) presents a striking contrast to the simple situation found when the cells of one region are plotted against those of another and indicates that the relative growth of two of these regions cannot be explained alone by the relative increase in the size of their cells. Also, the fact that one region may increase more rapidly than a second, while the cells of the second are enlarging more rapidly than those of the first, suggests that the relative rate of cell division may be a greater factor in the relative growth of these regions than is the relative enlargement of the cells. To study the relative growth



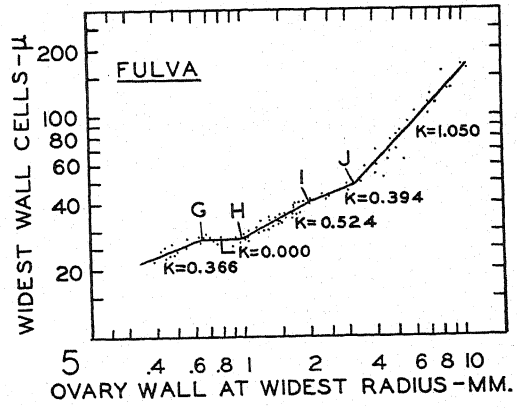
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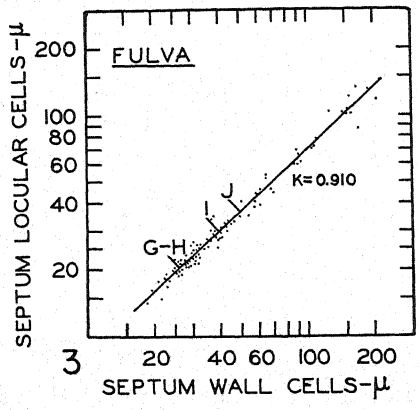
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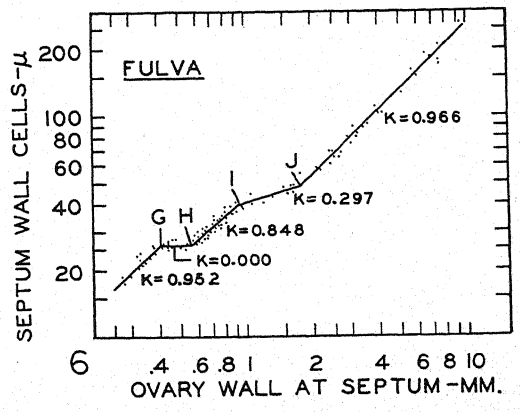
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6

FIGS. 1–6.—Relative growth curves of average diameter of cells: fig. 1, of ovary wall at widest radius and at midrib; fig. 2, of ovary wall at septum and at midrib; fig. 3, of locular region at septum and of ovary wall at septum; fig. 4, of ovary wall at midrib, and width of ovary wall at midrib; fig. 5, of ovary wall and its width at widest radius; fig. 6, of ovary wall and its width at septum. Each point represents average of thirty-five cells measured in direction parallel to radius in which they lie. In all figures, *G* occurs just before meiosis of megasporocyte and *H* just after; *I* occurs just at or just after beginning of fertilization and *J* very shortly after fertilization.

of a region and of the cells of which it is composed, the wall at the midrib and widest radius and the wall and locular region of the septum were plotted separately against the cells of those regions (figs. 4–7). The relation between cell size and organ size in *Iris* ovaries is not a simple one.

When a cell divides to form two, four situations may be found in the relationship of the size of the mother cell to that of the daughter cells, provided the two daughter cells behave in the same manner: (*a*) the daughter cells may not enlarge at all following the division, in which case they will together occupy the same volume as the original cell; (*b*) they may enlarge somewhat but each may not attain the length of the original cell; (*c*) each cell may enlarge to the size of the original cell; (*d*) each may become larger than the original cell after the division.

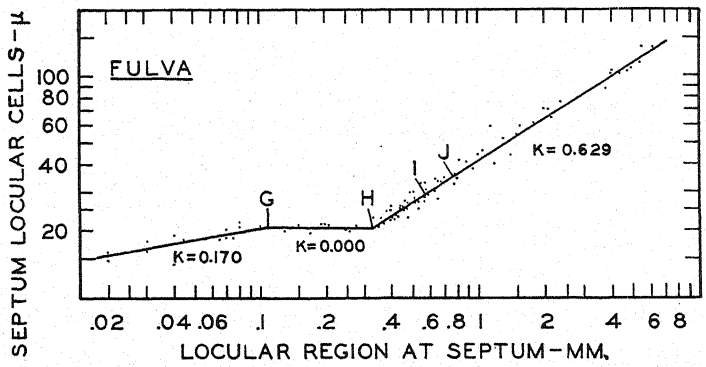


FIG. 7.—Relative growth curve of average diameter of cells of locular region of septum and width of this region.

In a cellular structure such as the ovary, if all the cells were behaving in approximately the same manner the relationship between cell size and organ size would differ according to the preceding situations. If ovary size were plotted against cell size, with ovary size as the abscissa and cell size as the ordinate, each situation would give a curve with a different slope. In the first case there would be continuous marked decrease in cell size unaccompanied by any increase or decrease in ovary size, and the relative growth curve would be a vertical straight line directed downward. The second situation would result in a decrease in cell size accompanied by an increase in organ size. If the percentage of decrease in cell size were the same after each division, the curve would be a straight line with a negative slope running from the upper left to the lower right. The slope of the curve would depend on the percentage of cell decrease. In the third case, when the daughter cells grow to the size of the parent cell after each division but grow no larger, there is an increase in ovary size without any change in cell size, and the curve is a horizontal straight line running from left to right. The fourth situation would mean an increase in both cell and ovary size; if the percentage of increase in cell size is the same after each division the curve will be an oblique straight line whose slope, k, will be between zero and one and will depend upon the extent of the increase in cell size between divisions. The greater the percentage of increase, the greater will be

the value of k and the greater will be the slope of the line. A fifth situation may also be found but it does not involve cell division. If there is an increase in cell size but not in cell number, ovary size will increase uniformly with cell size, the relative growth curve will be a straight line at a 45° angle, and the value of k will be one. A sixth situation could result from an increase in cell size accompanied by a decrease in cell number. This might occur if some of the cells disintegrated while the remaining ones enlarged. Some of the enlargement of the remaining cells would probably be used to fill up the space occupied by the disintegrated ones, so ovary size would not increase so markedly as cell size, and the curve would have a value of k greater than unity but would be a vertical line only if there were no growth in the ovary to accompany the increase in cell size.

These relationships obtain only if all the cells are acting in the same manner. If some cells were increasing in size while others were decreasing, or if the percentage of increase or decrease differed after each division, the results would probably be too complicated for analysis. In *I. fulva*, all the cells of the ovary wall appear to behave alike, or essentially so, and when cells increase in size following division they all do so, and the percentage of increase is the same for all cells of the wall at the same radius, even though the initial size is not the same. The thirty-five cells selected for measurement were chosen from all parts of the ovary wall at the radius under consideration. The cells of the inner part of the wall are the smallest, those of the outer part are usually the next smallest, while those of the middle region are generally the largest. This size difference might upset the results, except that in selecting the cells of a given radius they were chosen so that the inner, middle, and outer parts of the wall were represented in about the proportions of their thicknesses. In addition, if there is increase in cell size following division, it occurs in essentially the same degree in the inner, middle, and outer regions of the wall. The average of the thirty-five cells is therefore a fairly accurate estimate of the size of the wall cells in a given radius at a certain stage of development.

Of the six situations just listed, only the third, fourth, and fifth are found in *I. fulva;* the sixth situation, where cell and wall enlargement is accompanied by the disintegration of some of the cells, has been found in the related species *I. hexagona* var. *giganticaerulea* (unpublished data).

The third situation is found in the ovary wall at the midrib, widest radius, and septum, and in the locular region of the septum; this situation is observed when the megasporocyte is undergoing its two meiotic divisions (between points *G* and *H* in figures 4–7). During this period of ovary development the cells are dividing in all four regions, and the daughter cells grow only to the size of the original cells; therefore, all increase in wall size is due to cell division alone.

The fourth situation is found in a number of instances. In each case the slope is less than 45°, and its steepness depends on the extent to which the daughter cells enlarge after each division. For example, in the curve of the locular region of the septum and its cells, the amount of increase is slight before meiosis, for the value of k is 0.170. For the wall at the widest radius, the slope before meiosis is greater than that of the curve of the locular region, the value of k being 0.366. For the same part of the curve of wall at the midrib and its cells, k has a value of 0.881. Other regions of the

curves may also be found in which this situation exists.

The fifth situation is found in the last part of the curve of midrib wall and its cells; the corresponding part of the curves of the wall at the septum and its cells and of the wall and cells at the widest radius also probably fall into this category, although the value of k deviates in each case by about 0.05 from unity. In a general way these results are the same as those found by SINNOTT (5) in cucurbits. In *I. fulva*, however, the early part of the relative growth curve is more complex, and in the locular region of the septum cell division continues throughout development.

The development of the ovary wall as a whole presents a rather complicated picture. Before meiosis, the walls at the midrib and septum grow at almost the same rate, with the midrib wall growing just slightly faster; but the wall at the widest radius grows somewhat faster. At the same time the cells at the septum and midrib are growing at about the same rate, but those at the widest radius actually grow more slowly than those of the other two regions. More active cell division at the widest radius accounts for the more rapid growth of that region and is a greater factor in wall enlargement than is cell enlargement between mitoses. The interrelationships of these relative growth rates are seen in the part of the curve before point G in figures 1, 2, 4, 5, and 6.

During meiosis the cells in all three regions attain only the size of the parental cell after each division, and there is no cell enlargement. The more rapid growth of the wall at the widest radius when compared with the wall at the midrib and septum can be accounted for only by greater cell division. From meiosis until just before fertilization the widest radius continues to grow more rapidly than do the other two, which still grow at almost the same rate with reference to one another. Again, more active cell division must account for this more rapid growth, for the cells of the wall at the widest radius do not enlarge quite so much after each division as do those of the wall at the two other radii. This is shown by the fact that the value of k for this part of the curves (between points H and I) is only 0.524 for the widest radius but is 0.833 for the midrib and 0.848 for the septum.

During fertilization the wall at the widest radius grows much more rapidly than the wall at the midrib, and the wall at the septum grows still more rapidly than the wall at the midrib. The cells at the midrib wall, however, continue to grow more rapidly than those of the wall at the widest radius and at about the same rate as the cells of the wall at the septum. Again, more active cell division and the fact that the cells divide more frequently without growing so large between divisions account for the more rapid growth of the wall at the widest radius and at the septum. During this period (between I and J) the cells at the midrib have ceased to divide and are increasing in size only, as shown by the k value of 1.005 in figure 4. At the widest radius k is 0.394 and at the septum is 0.297 during fertilization, which indicates that the cells of these regions are enlarging but little after each division. After fertilization, cell division has ceased at the midrib and widest radius and has practically ceased at the septum. Since there is no increase in cell number during this period, all differences in relative growth rates are determined entirely by differences in relative enlargement of the cells. The cells at the midrib enlarge somewhat more rapidly than do those at

the widest radius ($k = 0.856$); similarly, the wall at the midrib is growing relatively somewhat more rapidly than that at the widest radius ($k = 0.839$). The midrib cells are growing just a little more rapidly than those at the septum ($k = 0.988$), but the septum wall is growing just a little more rapidly than the midrib wall ($k = 1.071$). This difference may be due merely to errors of sampling or may represent a real difference, for figure 6 indicates that there is possibly a small amount of cell division still going on at the septum after fertilization. All the k values in these curves are naturally subject to errors of sampling, but it was not considered worth while to determine their standard errors.

Before meiosis, the locular region at the septum grows much more rapidly than the wall at the septum ($k = 4.155$), although the cells of the wall are enlarging a little more rapidly than those of the locular region. Greater cell division at the locular region undoubtedly accounts for this difference in relative growth. The wall cells are dividing very infrequently, and almost all the growth of the wall is due to cell enlargement ($k = 0.952$); at the locular region almost all growth is due to cell division and the cells enlarge very little between divisions ($k = 0.170$). During meiosis there is no cell enlargement in either region, and after each division the cells attain the size of the parental cell and then divide again. The more rapid growth of the locular region when compared with the wall undoubtedly indicates much more active cell division there. Between meiosis and fertilization, the locular region grows just barely more rapidly than the wall, but the cells at the wall enlarge more rapidly than those of the locular region, indicating again somewhat more active cell division in the locular region. In both regions there is both division and enlargement of cells, and at the locular region there is less enlargement than at the wall. During fertilization the condition changes radically. Much more active cell division in the wall during this period, coupled with a slightly greater relative growth of the wall cells, makes the wall grow more rapidly than the locular region. After fertilization, cell division has almost ceased in the wall but is still active in the locular region, and therefore the locular region grows more rapidly than the wall ($k = 1.449$)—even though the wall cells enlarge somewhat more rapidly than the locular cells.

One of the most striking features of the relationship of cell number to cell size in these ovaries is that the rate of cell enlargement in one region in relation to another never changes throughout development. The rate of cell enlargement to growth of the region in which the cells are located may change several times during development, but such a change in one region is compensated for by a corresponding change in another. Consequently the relative growth rate of the cells of one region and those of another is determined as soon as the cells first divide and enlarge, and this relative rate is never changed during the entire growth of the ovary. All differences in the relative growth rate of two regions of the ovary are determined solely by changes in the relative extent to which mitosis occurs.

Summary

1. In *Iris fulva*, relative growth of the ovary wall at the midrib, widest radius, and septum, and of the locular region of the septum is a function of the relative enlargement and relative amount of multiplication of the cells of those regions.

2. In the earliest stages of development both cell enlargement and cell mul-

tiplication are taking place, and the relative amount of each may vary in different radii. During meiosis cell enlargement does not take place, and growth in all these regions is due to increase in cell number. From meiosis until just before fertilization, cell division occurs and there is cell enlargement following each division, but the amount of enlargement is not the same in each region. The same is true for the period during fertilization, except in the wall at the midrib where cell division has ceased and wall growth is due entirely to cell enlargement. After fertilization there is no further division of cells in the wall at the midrib and widest radius, and very little in the wall at the septum; but considerable division still occurs in the locular region of the septum.

3. Differences in the relative growth of two regions are due chiefly to differences in the extent of cell division in these regions, for greater cell enlargement in one region in relation to a second is frequently accompanied by relatively greater growth of the second region, and changes in the relative growth rates of two regions are not necessarily accompanied by corresponding changes in the relative enlargement of their cells.

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LITERATURE CITED

1. HOUGHTALING, H. B., A developmental analysis of size and shape in tomato fruits. Bull. Torr. Bot. Club **62**:243–252. 1935.
2. RILEY, H. P., Development and relative growth in ovaries of *Iris fulva* and *I. hexagona* var. *giganticaerulea*. Amer. Jour. Bot. **29**:323–331. 1942.
3. ———, Development of the embryo sac of *Iris fulva* and *I. hexagona* var. *giganticaerulea*. Trans. Amer. Micros. Soc. (In press.)
4. RILEY, H. P., and MORROW, DOROTHY, Cell size in developing *Iris* ovaries. Amer. Jour. Bot. **27**: 707 (abstract). 1940.
5. SINNOTT, E. W., A developmental analysis of the relation between cell size and fruit size in cucurbits. Amer. Jour. Bot. **26**:179–189. 1939.

EFFECTS OF PHOTOPERIOD ON MICROSPOROGENESIS IN BILOXI SOYBEAN

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 545

CHESTER S. NIELSEN

(WITH NINETEEN FIGURES)

Introduction

In the early work on photoperiodic response of many plants it was found that when they were returned to photoperiodic cycles not conducive to flowering, they frequently abscissed their flower primordia and became vegetative (3). In other instances the flowers tended to be sterile (8). *Xanthium pennsylvanicum*, which will initiate floral primordia and produce macroscopic flowers following only one photoinductive cycle, was found by NAYLOR (6) to produce inflorescences that differed markedly in both rate and nature of development according to the number of photoinductive cycles given.

NEIDLE'S work with *Xanthium* (7) indicated that both nitrogen supply and photoperiodic treatment were involved in the production of viable pollen.

GARNER and ALLARD (3, 4) found that when Biloxi soybeans were maintained on photoperiodic cycles of slightly less than 13 hours of light and 11 hours of darkness, flower primordia developed into flowers in which pollination took place, and fruits and seeds developed. If Biloxi soybeans were maintained on photoperiodic cycles of 8 hours of light and 16 hours of darkness, however, the primordia developed into small, often minute, flowers. Such flowers seldom formed full-sized pods, and few seeds were produced. In contrast with this, in plants held for a few days at the critical period or slightly below it until flower primordia were initiated and then transferred to a photoperiod longer than the critical, a few of the primordia continued to develop into flowers much larger than those on plants maintained at or below the critical. Development of carpels on such plants was slow and these often contained no seeds.

These studies suggest that some type of cytological disturbance might be associated with the length of the photoperiod the plants were subjected to, subsequent to photoperiodic induction. The present work deals with this and was undertaken to determine the effect of various light and dark treatments on microsporogenesis in Biloxi soybeans which had previously received different numbers of photoinductive cycles.

Procedure

One of the experiments reported here was conducted in the spring and summer of 1939, while the other two were conducted during the spring and autumn of 1941. In all three experiments environmental conditions up to the time of treatment were made as nearly identical as possible. Seeds were planted in $4\frac{1}{2}$-inch clay pots filled with a rich loam soil. The seeds were germinated on well-lighted greenhouse benches, where supplementary illumination from Mazda filament lamps extended the length of the photo-

period. This light, of approximately 75 foot-candles at the leaf surface, was supplied from sunset until 2:00 A.M., thereby affording a photoperiod of about 21 hours out of 24. This photoperiod of 21 hours of light and 3 hours of darkness was supplied from the time the first trifoliate leaf was beginning to expand until treatment was begun.

Just before treatment, the plants were selected for uniformity in size and vigor and divided into several lots, each of which (except the controls) received a specific number of photoinductive cycles (table 1). These photoinductive cycles of 8 hours of light and 16 hours of darkness were provided by drawing a double-thickness black sateen cloth over the light framework arranged over the bench at 4:00 P.M. and removing it at 8:00 A.M.

In the first and second experiments, each lot of plants, except the controls, received from three to nine photoinductive cycles before being returned to long photoperiod conditions of 21 hours of light and 3 hours of darkness. In the third experiment different lots of plants received from two to ten photoinductive cycles before being returned to long photoperiod conditions, which in this case were 16 hours of light and 8 hours of darkness.

Three types of controls were employed. One group of plants remained on long photoperiod until harvested. None of these ever flowered. The second group was maintained on natural photoperiod until harvested. The third group remained on short photoperiod until harvested.

Subsequent to photoinductive treatment, collections were made at regular intervals from each lot in each of the experiments, according to the procedure outlined in table 1. In each of the experiments collections were begun at progressively later dates. Thus in the first experiment collections were begun as early as 1 day after photoinductive treatment and were continued for more than 1 month. In the second experiment collections were begun 22 days after treatment had been given and continued for 2 months. Collections in the third experiment started 26 days after photoperiodic treatments were given and continued for more than $2\frac{1}{2}$ months.

To obtain material for cytological study, the main axis of the plant was cut into short segments, and each node bearing floral buds was placed in Sax's modification of Navashin's solution. The material was washed in tap water and run up in ethyl-tertiary butyl alcohol to paraffin. Sections were cut at 8–10 μ, stained with Heidenhain's iron-alum haematoxylin, and counterstained with orange G in clove oil.

Results

None of the plants in any of the experiments—with the exception of the control lots which received natural photoperiod and those which were maintained on photoinductive cycles—had opened their flowers by the end of any collecting period. In every instance the time permitted the plants to mature exceeded the time recorded by BORTHWICK and PARKER (1) as being sufficient for maturation following a specific number of photoinductive cycles. This difference in time required for blooming is probably attributable to the difference in length of photoperiod used following the photoinductive cycles, or to a difference in light intensity or quality at Beltsville, Maryland, and Chicago, Illinois.

Examination of the axillary buds of the control plants showed that the flower primordia first appear as enlarg-

ing protuberances in the axils of bracts along the axis. The stamen primordia appear while the petal primordia are still small. The latter grow very slowly until the microsporocytes, characterized by dense cytoplasm and a relatively large nucleus (as contrasted with somatic cells), are differentiated in the anthers (fig. 1).

otic metaphases and anaphases show the haploid number of chromosomes to be 20, which is in agreement with the reports of FUKUDA (2) and VEATCH (9). Anaphasic separation of the bivalent chromosomes follows quickly (figs. 5, 6), resulting in the formation of two nuclei which pass into an interphase of short duration (fig. 7). The second meiotic di-

TABLE 1

Lot no.	Age in cycles of long photoperiod at time of treatment			No. of cycles of short photoperiod			No. of cycles of long photoperiod following induction*					
							Experiment I		Experiment II		Experiment III	
	Experiment I	Experiment II	Experiment III	Experiment I	Experiment II	Experiment III	First plant	Last plant	First plant	Last plant	First plant	Last plant
1A	On cycles of long photoperiod until harvested; entirely vegetative											
1B	27	Control plants, on cycles of natural photoperiod, from 14 to 71 cycles until harvested										
2	.....	.....	25	.....	.....	2	.....	.....	.....	.....	59	80
3	27	23	25	3	3	3	9	26	29	61	58	79
4	27	22	25	4	4	4	8	31	29	61	52	73
5	28	22	25	5	5	5	7	35	30	52	43	64
6	29	22	25	6	6	6	5	33	29	51	40	61
7	32	22	25	7	7	7	1	30	26	47	35	56
8	33	22	25	8	8	8	1	29	25	46	28	50
9	34	24	25	9	9	9	1	36	22	43	27	49
10	.....	.....	25	.....	.....	10	.....	.....	.....	.....	26	48
11	27	On cycles of short photoperiod, from 13 to 30 cycles until harvested										

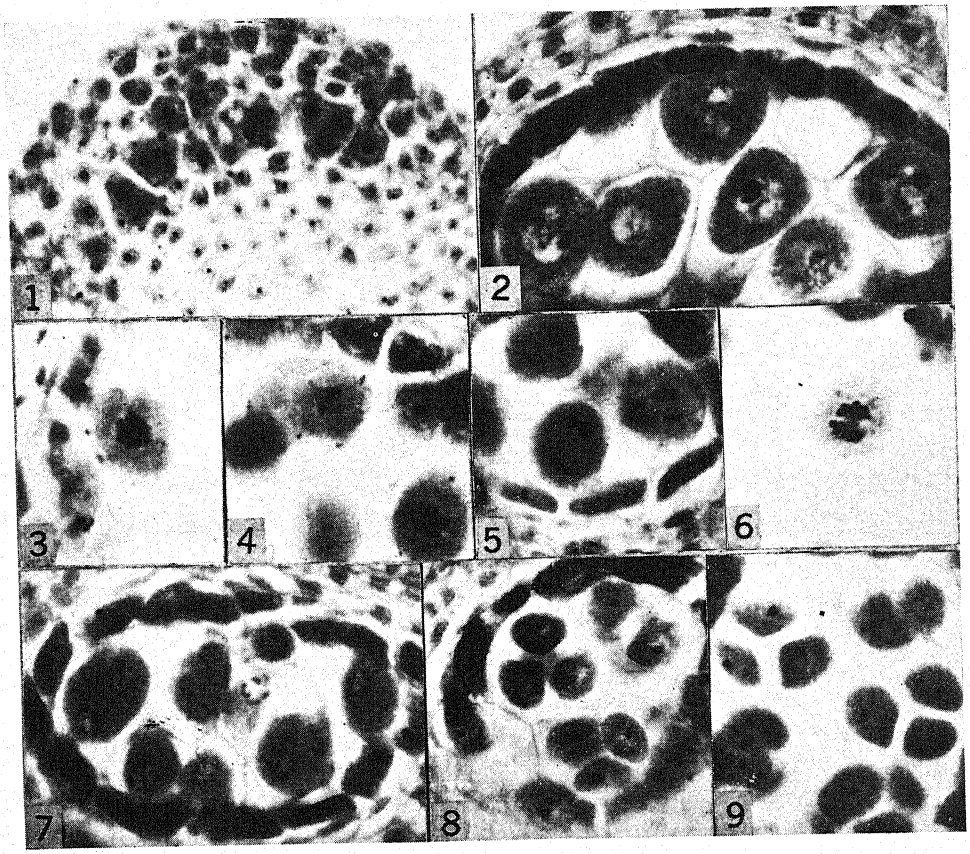
* Plants from each lot harvested progressively, so that material in various stages of development would be available. Number of cycles of long photoperiod which first and last plants of lot received after photoinduction is indicated.

Growth of the microsporocyte and its nucleus soon occurs. The characteristic reticulum of the metabolic nucleus is transformed into the slender thread stage of the leptotene. The threads appear as numerous elements, rather uniformly distributed throughout the nucleus. Nuclear diameter increases markedly as the synaptic pairing of the zygotene proceeds to the pachytene stage (fig. 2). Following diplotene and diakinesis, the bivalent chromosomes are next arranged on the equator of the spindle (figs. 3, 4). Repeated counts made at the first meivision produces the four-nucleate stage, which is soon followed by cleavage to form the tetrahedrally arranged quartet of microspores (fig. 8). Disintegration of the microsporocyte wall takes place as the microspores within it round up and their walls thicken (figs. 9, 10).

Examination of buds from the photoperiodically treated plants showed that flower primordia had been initiated and had developed essentially as in control plants, up to the differentiation of microsporocytes. Abnormalities in microsporogenesis now begin to appear, the time

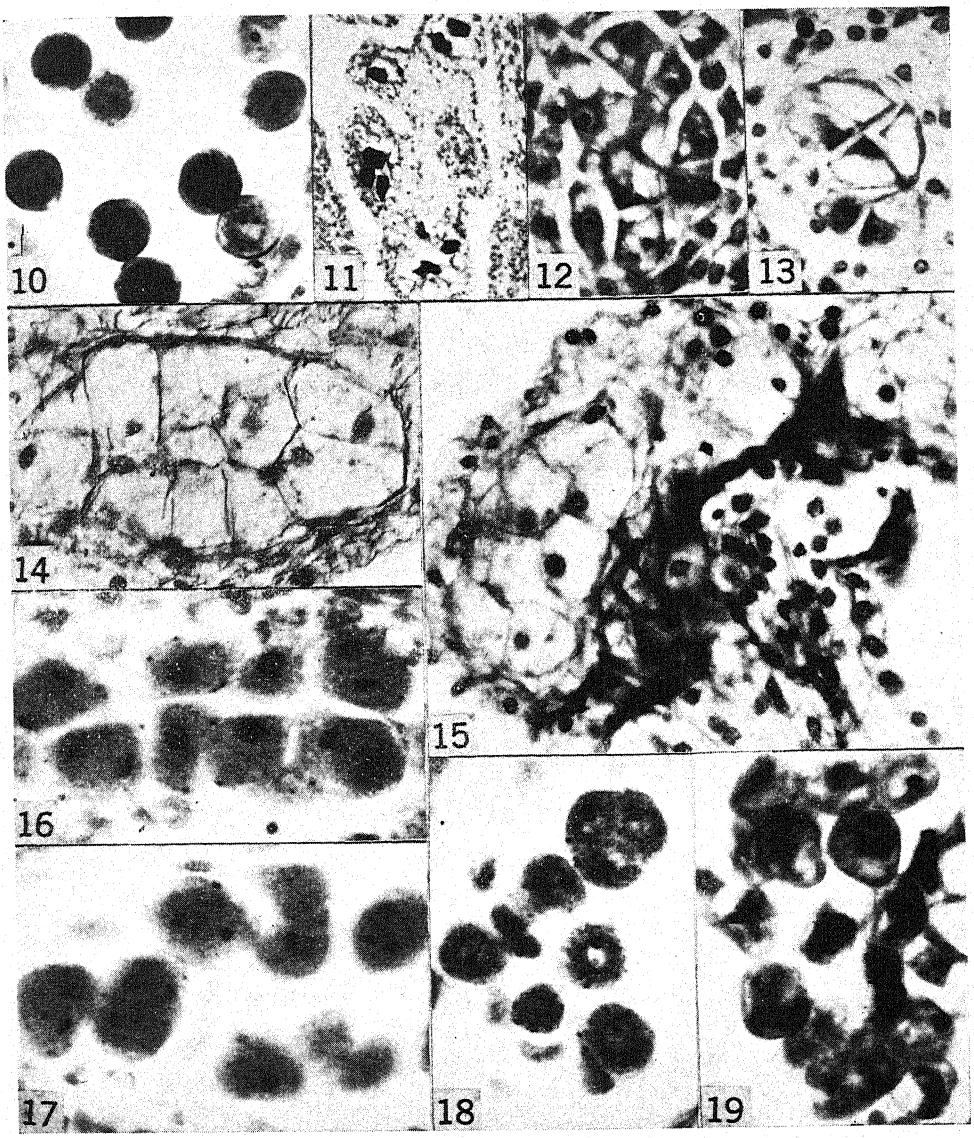
and degree depending in part upon the number of photoinductive cycles which the various lots of plants had received.

The first observable abnormality in the microsporocytes is a vacuolation of the cytoplasm. The vacuoles increase in size as the cells enlarge, and there appears to be little, if any, increase in volume of the cytoplasm or of the nucleus. Cell enlargement may terminate at any point before the size characteristic of normal microsporocytes is reached. Degenerative changes may set in while enlargement is proceeding, often resulting in irregular masses of disintegrating sporogenous tissue which stains intensely with haematoxylin (fig. 11). On the other hand, enlargement and vacuolation may continue, resulting in large cells with little cytoplasm and small nuclei. Successive stages in this process are shown in figures 12 and 13. The last stages are represented by shriveled anthers, the loculles of which contain cells with scanty cytoplasm and whose nuclei are either small and degenerated or occasionally entirely lacking (fig. 14). Shortly afterward the anther wall begins to break down, and eventually the entire stamen collapses (fig. 15).



FIGS. 1–9.—From control plants of lot 1B: Fig. 1, young microsporocytes; fig. 2, pachytene; figs. 3, 4, metaphase I; figs. 5, 6, anaphase I; fig. 7, interkinesis; figs. 8, 9, quartets.

In the lots receiving fewer than six cycles of short photoperiod, degenera-



FIGS. 10–19.—Fig. 10, normal microspores from plant of lot 1B. Fig. 11, shriveled anther containing degenerated miscrosporocytes, lot 6. Figs. 12, 13, microsporocytes showing vacuolation and early degeneration, lot. 8. Fig. 14, locule of anther with enlarged microsporocytes containing little cytoplasm and unenlarged nuclei, lot 6. Fig. 15, stamen showing early stages of degeneration of wall cells, lot 8. Fig. 16, anaphase I, lot 8. Fig. 17, anaphase II, nuclei irregular and abnormal, lot 8. Figs. 18, 19, microspores showing various stages of degeneration, lot 8.

tion of the microsporocytes at early stages of microsporogenesis is characteristic. In only one instance was morphologically normal microspore development observed. A few plants given six cycles of short photoperiod have shown development beyond the microsporocyte stage. The meiotic process may proceed apparently normally through the first meiotic division (fig. 16) and as far as the anaphases of the second (fig. 17). In many cells there is frequently evidence of chromosomal degeneration at these stages, with consequent failure of telophasic reorganization. Some cells in which an apparently normal telophasic reconstruction occurs may undergo premature cleavage, followed by collapse of the resulting abnormal microspores. Normal meiosis does occur in the microsporocytes of some anthers of plants which had received six or more photoinductive cycles, and tetrads are formed which appear capable of developing into functional microspores (fig. 19). A high percentage of the microspores in these plants undergo degeneration, however, even at these advanced stages of development.

Differences in degenerative changes between experiments I and II, which had received postinductive photo-periods of 21 hours, as compared with those of experiment III, receiving 16 hours, are quantitative rather than qualitative, in that abnormalities of the same type occur in all but are more intense in the first two. In all the experiments, regardless of the character of the postinductive photoperiods, less than six inductive cycles of short photoperiod result in suppression of microsporogenesis prior to the formation of microspores. With six or more inductive cycles, apparently normal as well as abnormal pollen was produced, with an increase in percentage of the former as the number of inductive cycles approaches ten. Nevertheless, in the lot receiving ten cycles of short photoperiod, although degeneration in earlier stages was less, disintegration of microspores and the presence of nonviable pollen is still apparent.

Discussion

The degenerative changes observed during microsporogenesis in Biloxi soybean which had been given fewer than six photoinductive cycles and then returned to a postinductive photoperiod of 16 or 21 hours have been found to be similar, in many respects, to the effects of carbohydrate deficiency in the tomato as described by HOWLETT (5). He found that severe carbohydrate deficiency resulted in failure of the nuclei of sporogenous cells to reach meiotic divisions. The walls of these cells collapsed and the protoplasts degenerated before the prophases were initiated. This occurred concurrently with retardation in development of the entire flower as a result of limitation of carbohydrates.

Early degeneration of the sporogenous tissues likewise occurred in all the groups of photoperiodically induced soybean plants used in these experiments, but it was only in those groups receiving five or fewer photoinductive cycles that development failed to proceed beyond the early prophase stages of meiosis. And even in these groups the perianth segments were not noticeably retarded in development up to this time.

In the groups given six to ten photoinductive cycles, degeneration at later stages in meiosis was frequent. In some anthers, microsporocyte enlargement occurred regularly; but during its progress increasing vacuolation of the cytoplasm, accompanied with nuclear disintegration, resulted in the production of shriveled

anthers containing disorganized, densely staining masses of sporogenous cells. In other anthers the sporocytes appeared to undergo an apparently normal meiosis and produced four groups of chromosomes, each inclosed by a nuclear membrane. In those plants given seven or eight photoinductive cycles, degeneration frequently occurred before the four groups of chromosomes were reorganized as resting nuclei and before cleavage into microspores took place. Even when resting nuclei were organized, as was often observed in plants given nine or ten photoinductive cycles, degeneration of the microspores might still occur immediately following cleavage. Morphologically normal microspores were found, however, in many of the plants which had received eight to ten photoinductive cycles, despite the high percentage of degeneration in earlier stages of meiosis.

As in carbohydrate-deficient tomato plants, where the degree of degeneration was directly proportional to the severity of the deficiency and to the level of development at which it is operative, the number of photoinductive cycles in the soybean influences similarly the degree and stage to which normal development of microsporocytes may proceed. The length of the postinductive photoperiod appears also to be an important factor affecting the degree of sterility in the plants of these experiments, when they are compared with the results obtained by BORTHWICK and PARKER, who used natural photoperiod following the photoinductive treatments.

Summary

1. Three experimental series of Biloxi soybeans were given photoinductive treatments of from two to ten cycles, consisting of 8 hours of natural daylight followed by 16 hours of darkness. After induction, series I and II were placed on cycles of long photoperiod, consisting of 21 hours of light and 3 hours of darkness, and series III on cycles of 16 hours of light and 8 hours of darkness.

2. Three types of controls were employed. One group of plants was kept on long photoperiod. None of these flowered. A second group was kept on natural photoperiod and a third on short photoperiod. These two groups flowered.

3. Floral buds from controls and from the three experimental series were collected progressively in order to obtain various stages in development. In the second and third groups of controls, floral development was normal and meiosis occurred as has been described by previous workers. In all the experimental groups the floral structures developed normally until the differentiation of sporocytes had occurred in the anthers. Following this, various abnormalities in meiosis were observed.

4. In plants given five or fewer photoinductive cycles, the sporocytes might begin to enlarge, accompanied by marked vacuolation of the cytoplasm and ultimate disintegration of their nuclei. Only a few of the sporocytes ever reached the metaphase stage of meiosis I.

5. In plants receiving six or more photoinductive cycles of short photoperiod, degeneration of microsporocytes in some anthers may occur as already described, but in others the sporocytes undergo apparently normal meiosis, and four groups of chromosomes are formed. In some instances degenerative changes occur at this point before cleavage into microspores takes place; in others cleavage occurs followed by degeneration before development of normal microspores has been accomplished; while in a very small percentage of instances apparently normal microspores are formed. Even

those plants receiving as many as ten photoinductive cycles of short photoperiod showed a high percentage of degenerated microspores.

6. The long postinductive photoperiod employed in these experiments appears to have played a significant role in bringing about the degenerative changes observed and in suppressing the development of mature flowers.

The writer is indebted to Dr. J. M. BEAL for his counsel and assistance during the course of this investigation.

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LITERATURE CITED

1. BORTHWICK, H. A., and PARKER, M. W., Influence of photoperiods upon the differentiation of meristems and the blossoming of Biloxi soybeans. BOT. GAZ. **99**:825–839. 1938.
2. FUKUDA, Y., Cytogenetical studies on the wild and cultivated Manchurian soybeans (*Glycine* L.) Jap. Jour. Bot. **6**:489–506. 1933.
3. GARNER, W. W., and ALLARD, H. A., Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour. Agr. Res. **18**:553–606. 1920.
4. ———, and ———, Further studies in photoperiodism: the response of the plant to relative length of day and night. Jour. Agr. Res. **23**:871–920. 1923.
5. HOWLETT, F. S., The effect of carbohydrate and of nitrogen deficiency upon microsporogenesis and the development of the male gametophyte in the tomato, *Lycopersicum esculentum* Mill. Ann. Bot. **50**:767–804. 1936.
6. NAYLOR, FRANCES L., Effect of length of induction period on floral development of *Xanthium pennsylvanicum*. BOT. GAZ. **103**:146–154. 1941.
7. NEIDLE, EDITH K., Nitrogen nutrition in relation to photoperiodism in *Xanthium pennsylvanicum*. BOT. GAZ. **100**:607–618. 1938.
8. SHAFFNER, J. H., The influence of relative length of daylight on the reversal of sex in hemp. Ecology **4**:323–334. 1923.
9. VEATCH, C., Chromosomes of the soybean. BOT. GAZ. **96**:189. 1934.

CYTOLOGY OF APOGAMY IN POLYSTICUM TSUSSEMENSE

SISTER MARY THOMASINE PATTERSON

(WITH NINE FIGURES)

Introduction

Although numerous investigators have reported apogamy in various species of ferns, cytological investigations of the phenomenon have not been numerous. The first work of this nature was that of FARMER *et al.* (7), who found the number of chromosomes in the nuclei of the sporophytic cells to be twice that of those in the gametophytic cells in *Nephrodium pseudo-mas* var. *polydactyla*. They concluded that the doubling process occurred when nuclei of adjacent gametophytic cells fused. The resulting gametophytic cells, with the doubled number of chromosomes, produced the diploid sporophyte.

DIGBY (2), investigating *Nephrodium pseudo-mas* var. *cristata apospora*, found no change in chromosome number in passing from the gametophytic to the sporophytic generations.

FARMER and DIGBY (8) investigated the phenomenon in several ferns. These differed among themselves with respect to nuclear fusions. As a result of their investigations they made two divisions of apogamous ferns: those in which fertile spores were produced and in which there were nuclear migrations and fusions in the gametophytic phase and those which were both apogamous and aposporous and showed no change in chromosome number during the life cycle.

ALLEN (1) described a fusion of nuclei and cells in the sporangium of *Aspidium* (*Cyrtomium*) *falcatum*. The development of the sporangium was normal up to the formation of the sixteen sporogenous cells. These cells, according to her description, then fused in pairs and produced eight spore mother cells with the double number of chromosomes. The fusion was always in pairs and generally all the cells in the sporangium fused. Reduction divisions followed, and thirty-two haploid spores were formed.

STEIL (13) found that there were only eight sporogenous cells in the spore sac of *Nephrodium* (*Dryopteris*) *hirtipes*. When these cells and their nuclei were apparently about to form the usual sixteen cells, interruptions occurred at various stages which STEIL (13) described as incomplete nuclear and cell divisions. The subsequent meiotic divisions were regular, and haploid spores were formed which upon germination produced the gametophytes.

HAYES (9) found binucleate cells in the gametophyte of *Pellaea atropurpurea*. These cells resulted from nuclear division unaccompanied by cell division or from the migration of nuclei as described by FARMER and DIGBY (8). However, she never found fusion of these nuclei and was unable to trace the origin of the apogamous embryo to a "fusion" nucleus. The nuclei of both gametophytic and sporophytic cells contained forty chromosomes, so it was concluded that there was no cytological significance in the binucleate condition. She suggested that these binucleate cells might have a nutritive function similar to that of the binucleate tapetal cells of plants.

DÖPP's (3) findings with *Aspidium*

[Botanical Gazette, vol. 104

(*Dryopteris*) *remotum* were much the same as those reported by STEIL (13) for *Nephrodium* (*Dryopteris*) *hirtipes*. All the sporogenous cells did not behave in the same manner, however. He reported incomplete nuclear and cell divisions in many cases. In others some of the nuclei and cells divided completely but in an irregular manner, so that the resulting nuclei and cells were not equal in size. Consequently the sporangium contained from eight to sixteen spore mother cells of varying size, depending upon the number of cells that underwent the irregular divisions and the number of chromosomes each received, A third type of division was entirely regular, so that the sporangium contained sixteen spore mother cells, which later, however, produced sterile spores. The spore mother cells formed by either the incomplete or the irregular divisions gave rise to spores by meiotic divisions. The percentage of viability of the spores was good, for the most part. DÖPP (4) also found essentially the same phenomena in *Aspidium* (*Dryopteris*) *filix-mas* var. *crist.*

HEUN (10), investigating *Pteris cretica* var. *albo-lineata*, found that both gametophyte and sporophyte possessed the haploid number of chromosomes. He observed no fusion of nuclei or cells in either gametophyte or sporophyte, no incomplete nuclear and cell divisions of the sporogenous cells, and no reduction divisions in the spore mother cells.

STEIL (14) has reviewed the entire question of apogamy, and DÖPP (5) has summarized nuclear behavior in apogamous ferns, with special reference to incomplete divisions.

This investigation was undertaken to discover the nuclear history of the apogamous fern, *Polysticum tsussemense* J. Smith, including detailed studies of both gametophyte and sporophyte.

Material and methods

GAMETOPHYTE

Spores of *Polysticum tsussemense* J. Smith were obtained from plants grown in the greenhouse of Marquette University and sown on Beyerinck's solution, as modified by MOORE (12), in Erlenmeyer flasks, the nutrient solution and flasks having been sterilized previously in the autoclave. The flasks were kept in a Wardian case in the greenhouse. Three hundred and fifty-five sowings were made over a 2-year period. Gametophytes in all stages of development were fixed in chromo-acetic solution. The material from each fixation was separated into two parts. One portion was used for whole mounts, stained in Harris' haematoxylin, run up into Venetian turpentine, and finally into balsam. The other was dehydrated and infiltrated in isoamyl alcohol[1] and imbedded in paraffin. Sections were cut at 7 or 10 μ and stained with safranin, gentian violet, and orange G or fast green, or with only safranin and fast green.

To determine the percentage of germination of the spores they were sown on the surface of sterilized Beyerinck's solution, which had been placed into Stender dishes. Spore counts from individual sporangia were made by mounting the sporangia in water and then dehydrating them with glycerin. When the sporangia opened and the spores were liberated, the number could be readily counted. Immature spores were also counted after staining, according to MCCLINTOCK'S (11) smear method.

SPOROPHYTE

Material for the sporophytic studies was obtained from the same plants.

[1] First used in the biological laboratories of Marquette University by Dr. STEIL. The results of the use of this reagent have not yet been published.

Young sori in various stages of development were removed from the leaves and fixed in Flemming's medium fluid or in Navashin's fixative. Also portions of the pinnae with sori were fixed in the same solutions. Fixations were made at various times during the day for a period of 2 years. Various dehydrating and infiltrating agents were tried, but since isoamyl alcohol proved so satisfactory it was used most extensively. After imbedding in paraffin, sections were cut at 10 μ. Flemming's triple stain was generally used, although safranin and fast green were found satisfactory.

The number of spore mother cells in a single sporangium was determined from a study of serial sections of the sporangium and also by examination of smear preparations.

Chromosome counts were made from smears prepared according to Belling's technique as modified by McCLINTOCK (11). Instead of dehydrating with absolute alcohol and xylol and mounting into balsam, however, the material was mounted in euparol directly from absolute alcohol. Counts were made of sectioned material also. Dividing nuclei of cells of root tips, croziers, and sporogenous cells were obtained for the chromosome counts.

Observations

GAMETOPHYTE AND APOGAMOUS EMBRYO

The spores possessed a low percentage of viability and were rather slow in germinating. The first evidence of germination was the breaking of the exosporium and the extruding of the first prothallial cell. Chloroplasts, bright green in color, were abundant in this cell almost as soon as it emerged, and even before cross walls could be seen. The first division resulted in a projecting colorless rhizoid and a rather large cell containing many chloroplasts. The rhizoid elongates but undergoes no division. The chlorophyllose cell gives rise to a filament of six to eight cells by transverse divisions. From the filament, two, three, or more rhizoids are later produced.

After the formation of the filament of several cells the young prothallium becomes broadened at the anterior end, and soon the characteristic heart-shaped form of the gametophyte can be recognized. The entire plant is only one cell in thickness at this stage. Numerous one-celled glandular hairs are produced from the margins and on the dorsal surface. Further development results in a thickening of the central region of the prothallium and the formation of a typical cushion. Numerous long rhizoids are formed in the posterior region on the ventral surface.

When the plants were grown in strong light and in uncrowded cultures, no antheridia were produced. When light was weak, and especially if the plants were crowded, long filaments rather than heart-shaped gametophytes were formed, and antheridia with mature antherozoids were produced in abundance. Archegonia were not observed on any of the prothallia examined. Hundreds of prothallia were examined, both in sections and in whole mounts, to determine whether the cells of the cushion were binucleate, but at no time were cells of this type found.

While still small, less than $\frac{1}{8}$ inch in diameter, the gametophytes exhibit the first evidence of apogamy. A group of very small cells appears a short distance behind the apical notch. Later tracheids can readily be seen in this region. In sections the tracheids are seen to be bounded on either side by cells that are much

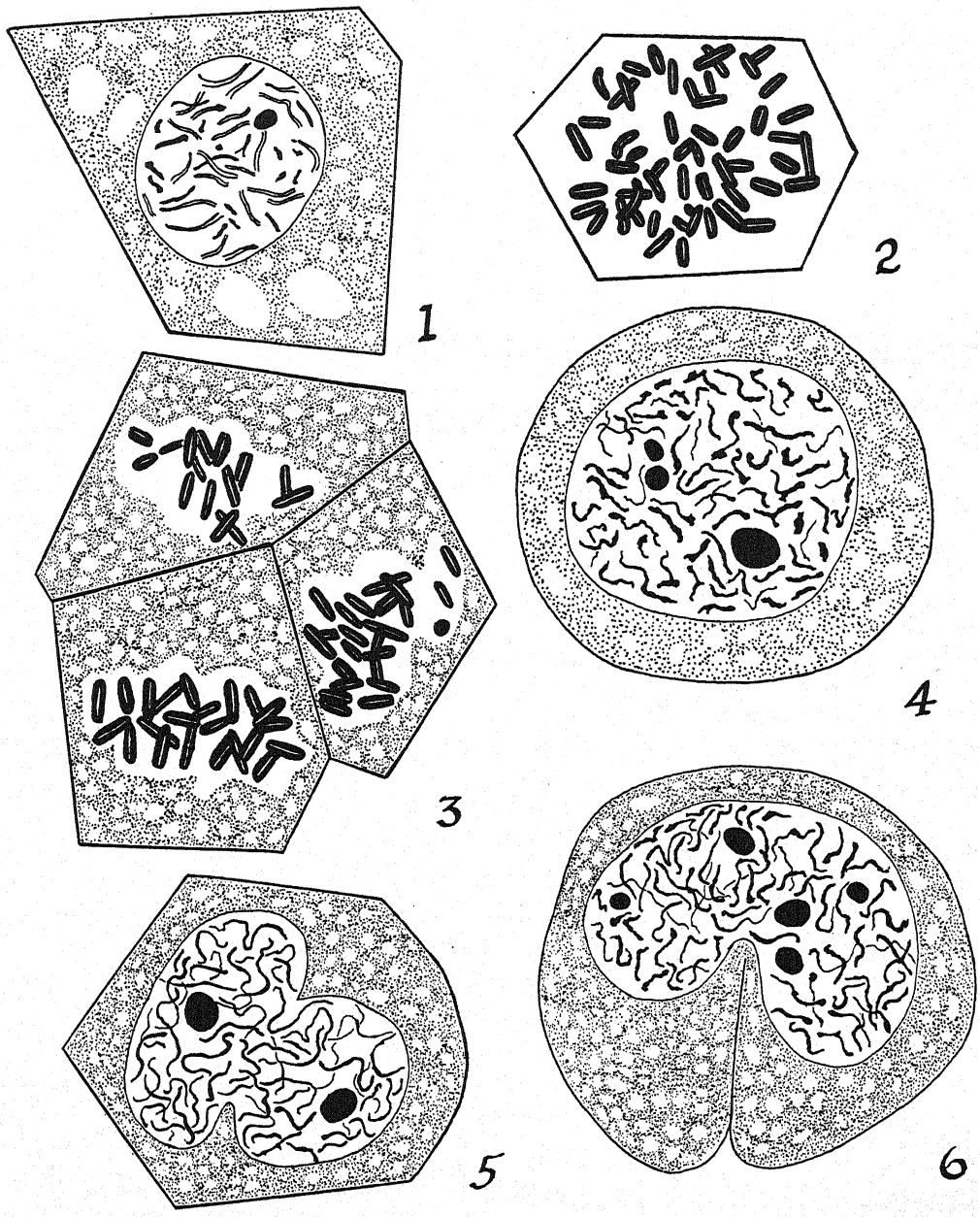
smaller than others of the gametophyte. These small cells have nuclei that are larger, in proportion to the size of the cells, than the nuclei of the larger cells. Soon after the formation of the tracheids in the gametophyte, the leaf, root, and stem regions are differentiated. These primordia give rise to the leaf, root, and stem, which are readily seen with the naked eye when the gametophyte is 2–3 months old. About 7 months after sowing the spores, the young sporophyte has produced three or four juvenile leaves. By this time the gametophyte begins to show evidence of dying, and the sporophyte continues an independent existence.

Sporogenous cells

Nothing unusual was observed in the development of the sporangium from the time of the appearance of the sporangium initial to the formation of the eight angular sporogenous cells. The events following their formation, however, do not proceed in the usual manner. The first evidence of irregularities in these cells is observed during the prophase of the nuclear division. Emerging from the metabolic condition the nucleus contains a large number of threads (fig. 1), which gradually contract and take a heavier stain. Each consists of two portions or a split chromosome. These lie scattered within the nucleus. Most frequently, this division proceeds no further and the nucleus becomes very large and spherical, almost completely filling the cell. It then passes directly into the metabolic stage again. It differs from the previous metabolic nucleus in its larger size and in the possession of the double number of chromosomes.

Often the divisions proceed further, the nuclear membrane disappears, and the split chromosomes lie free in the cell without any particular orientation (fig. 2). Spindle fibers may not be formed, and the chromosomes may not be arranged in an equatorial plate (fig. 3). Since no spindle fibers are produced, the chromosomes are not pulled apart. A new nuclear membrane is formed about the chromosomes, and each sporogenous cell, now destined to function as a spore mother cell, contains a large spherical nucleus with the diploid number of chromosomes (fig. 4). The cells are still quite angular in shape and in close contact with one another. The outstanding visible change is in the size of the nucleus, which now occupies almost the entire cell (cf. figs. 1 and 4).

Generally all eight cells of the sporangium are in the same stage of development and show the same evidence of incomplete nuclear division. Not infrequently, however, the nuclei, instead of being spherical in form, may be dumb-bell- (fig. 5) or kidney-shaped (fig. 6). These are the result of a division which was not interrupted in the prophase but at a later stage. Instead of the split chromosomes being scattered about with no particular orientation within the cell, they come to lie in the equatorial plate. Spindle fibers are weakly developed; hence the chromosomes are not drawn to the poles of the spindle, and diploid nuclei, some of which are at first irregular in shape, are formed. When some of the chromosomes are drawn to the spindle poles, the anaphase is partially initiated and the diploid restitution nucleus may be irregular in form, such as kidney- or dumb-bell-shaped, as a result of aberrations of the mitotic figure. Often the chromosomes do not reach the poles simultaneously; instead there are a number at each pole and the remainder scattered about on the spindle, some of them still being at the center of the first spindle.



FIGS. 1–6.*—Fig. 1, archesporial cell in early prophase. Fig. 2, chromosomes of archesporial cell lying free, showing no particular orientation. Spindle fibers failed to develop. Fig. 3, three of the eight archesporial cells with dividing nuclei. Fig. 4, diploid spore mother cell showing large spherical nucleus. Fig. 5, dumb-bell-shaped nucleus in spore mother cell, formed as result of incomplete division which proceeded through prophase. Fig. 6, kidney-shaped nucleus in spore mother cell, also formed by division interrupted at stage later than prophase; incomplete cell division also evident.

* All figures are camera lucida drawings made at ×1900 diameters. Figs. 8 and 9 from smear preparations; all others from sectioned material.

When the division is interrupted at a late mitotic stage, incomplete cell division often also occurs (fig. 6). The cell plate is laid down on the spindle fibers, but since these are frequently present on only one side of the nucleus, the cell plate does not extend completely across the cell (fig. 6).

As already stated, nuclei of different forms may be observed in the same sporangium, but the majority are of the large spherical shape which resulted from interruption of the nuclear division in the prophase.

After the incomplete nuclear division has occurred, often accompanied by incomplete cell division, the resulting cells gradually lose their angular shape and assume the large spherical form characteristic of spore mother cells. At the same time the tapetal cells begin to disintegrate and the spore mother cells separate. Somewhat later, most of the nuclei also assume the large spherical form, and generally there is no apparent evidence of the incomplete division.

Soon after the rounding up of the spore mother cells and their nuclei, the meiotic divisions are initiated. The chromosomes present in the double number, owing to the incomplete division, become long and thin during early prophase. The sister chromosomes then pair and pass into synapsis. The synaptic mass lies near one side of the nuclear membrane, and the rest of the nucleus appears as a large clear space. The synaptic knot loosens, and the chromosomes, in the paired condition, thicken and lie scattered about in the nucleus.

At diakinesis the chromosomes are easily observed in pairs. Passing into metaphase, the nuclear membrane disappears and the chromosomes lie in the equatorial plate. Counts made at this stage showed 91 pairs of chromosomes present. Spindle fibers are formed and are not difficult to demonstrate at this stage. The four chromatids characteristic of the meiotic chromosomes could be readily observed in the paired chromosomes (fig. 7). The doubleness of each of the chromosomes could also be demonstrated in the anaphase of the first or heterotypic division.

The second division follows almost immediately, and it is not unusual to find one nucleus beginning the second division while the other has already completed it. As a result of the meiotic divisions each cell of the spore tetrad contains the same number of chromosomes as seen in the sporogenous cells before the incomplete nuclear divisions occurred. The cells of the tetrad separate and finally round up to form mature spores.

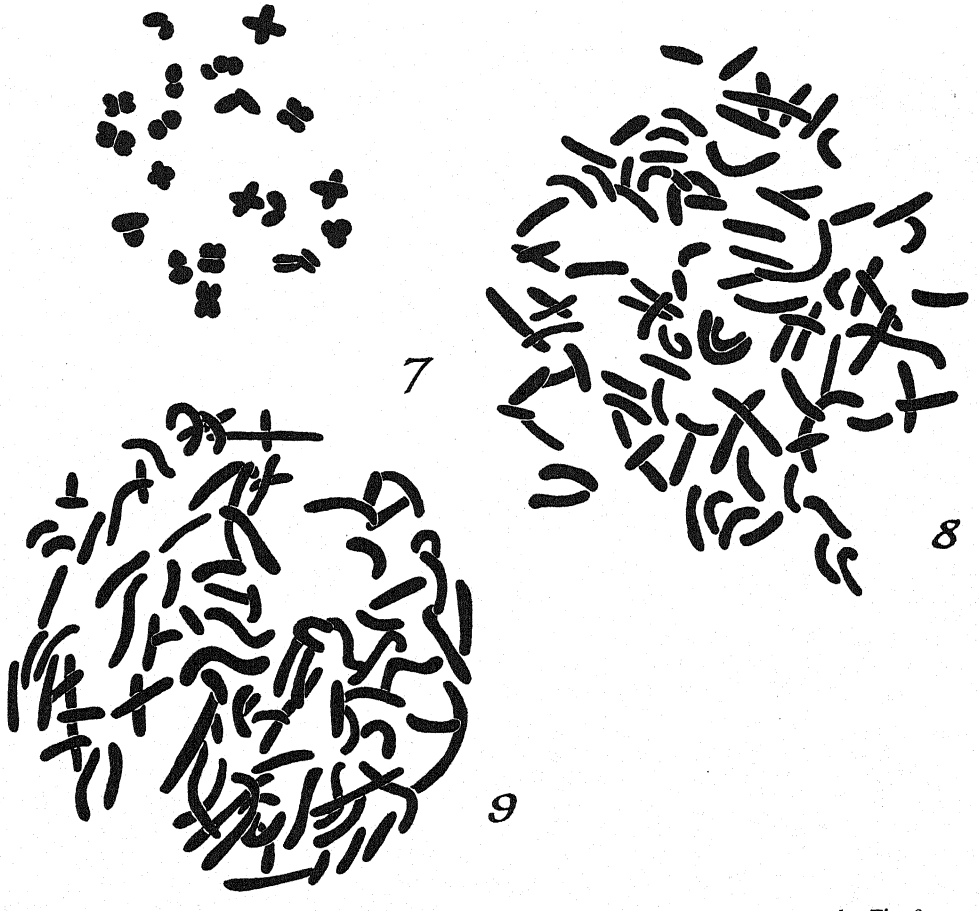
The relationship of chromosome numbers in the various phases in the life history of this fern is not, as has been demonstrated, the one typical of gametophytic and sporophytic generations. Since fertilization does not occur in the life history, the number of chromosomes in the gametophytic cells is carried over to the sporophytic cells. The number is doubled as a result of the incomplete nuclear division, which occurs in the sporogenous cell and is restored by the meiotic divisions which follow. Chromosome counts verified this fact. On account of the large number of chromosomes, however, the counts were made with considerable difficulty. The chromosome number obtained in the gametophytic cells (fig. 8), in the dividing nuclei of spermatogenous cells, and in root-tip cells (fig. 9) is approximately 91.

The sporophyte produces sori in great numbers. The origin and growth of the sporangia and sporogenous cells follow the usual pattern up to the eight-celled stage. Then the nuclei of the eight cells

undergo an incomplete division. This division is generally arrested during the prophase, but may proceed to metaphase or partial anaphase. As a result of the incomplete nuclear division, unaccompanied by cell division, the sporangium contains eight spore mother cells, each with the doubled number of chromosomes. These cells undergo meiotic divisions to form thirty-two spores, each with the haploid number of chromosomes.

The cytological history of *P. tsussemense* is similar to that first described by Steil (13) in *Nephrodium* (*Dryopteris*) *hirtipes* and to that in *Aspidium* (*Dryopteris*) *remotum* and in *A.* (*D.*) *filix-mas* var. *cristata* described by Döpp (3, 4). In the majority of cases, however, the ordinary mitotic divisions in *P. tsussemense* do not proceed in the eight sporogenous cells further than prophase, whereas in the other three species division is frequently interrupted at a later mitotic stage.

There was no evidence of fusion of nuclei, either in the gametophytic or in the sporogenous cells, like that described



Figs. 7–9.—Fig. 7, a few of the chromosomes of spore mother cell showing some tetrads. Fig. 8, gametophytic cell with 91 chromosomes. Fig. 9, cell of root tip with 91 chromosomes.

by FARMER and DIGBY (8) in *Lastrea pseudo-mas* var. *polydactyla* or in *Aspidium* (*Cyrtomium*) *falcatum* as reported by ALLEN (1).

Summary

1. The prothallia of *Polysticum tsussemense* J. Smith develop normally and produce antheridia but no archegonia.

2. The sporophyte always arises apogamously from cells of the gametophyte posterior to the apical notch.

3. Nuclear divisions of the eight sporogenous cells are generally interrupted during prophase but may proceed to metaphase or to partial anaphase. When nuclear division proceeds to partial anaphase, it is often accompanied by incomplete cell division.

4. The eight spore mother cells produced as a result of the incomplete nuclear divisions possess the diploid number of chromosomes, 91 pairs.

5. Meiotic divisions of spore mother cells are regular and give rise to 32 spores, each with 91 chromosomes.

6. The number of chromosomes in the gametophyte and sporophyte is therefore the same, 91.

The writer wishes to express her deep appreciation to Dr. W. N. STEIL of Marquette University for suggesting the problem and directing the investigation, and for his unfailing assistance and encouragement.

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LITERATURE CITED

1. ALLEN, R. F., Studies in spermatogenesis and apogamy in ferns. Trans. Wis. Acad. Sci., Arts, and Lett. 17:1–44. 1911.
2. DIGBY, L., On the cytology of apogamy and apospory. Preliminary notes on apospory. Proc. Roy. Soc. 76B:464–468. 1905.
3. DÖPP, W., Die Apogamie bei *Aspidium remotum*. Planta 17:86–153. 1932.
4. ———, Weitere Untersuchungen an apogamen Farnen. I. *Aspidium filix-mas* var. *crist* Hort. Ber. deutsch. Bot. Ges. 51:341–347. 1933.
5. ———, Karyologie. Manual of pteridology by VERDOORN. Pp. 253–283. 1938.
6. FARLOW, G. W., An asexual growth from the prothallium of *Pteris cretica*. Quart. Jour. Mic. Sci. 14:266–272. 1874.
7. FARMER, J. B., MOORE, J. E. S., and DIGBY, L., On the cytology of apogamy and apospory. I. Preliminary note on apogamy. Proc. Roy. Soc. 71:453–458. 1903.
8. FARMER, J. B., and DIGBY, L., Studies in apospory and apogamy in ferns. Ann. Bot. 21:161–199. 1907.
9. HAYES, M., Some studies of apogamy in *Pellaea atropurpurea*. Trans. Amer. Mic. Soc. 43:119–135. 1924.
10. HEUN, ALPHONSE, The cytology of apogamy in *Pteris cretica* Linn. var. *albo-lineata* Hort. Bull. Torrey Bot. Club 66:549–562. 1939.
11. MCCLINTOCK, B., A method for making acetocarmin smears permanent. Stain Technol. 4:53–56. 1929.
12. MOORE, G. P., Methods for growing pure cultures of algae. Jour. Appl. Mic. 6:2309–2314. 1903.
13. STEIL, W. N., A study of apogamy in *Nephrodium hirtipes*. Ann. Bot. 33:109–132. 1919.
14. ———, Apogamy, apospory, and parthenogenesis in the pteridophytes. Bot. Rev. 5:433–453. 1939.

EFFECTS OF GROWTH SUBSTANCES ON RESERVE STARCH

S. C. BAUSOR

(WITH EIGHT FIGURES)

As a sequel to work on the interaction of growth substances and soluble carbohydrates (1), a study was made of the effect of indoleacetic and β-naphthoxyacetic acids on the hydrolysis of starch. Previous reports have shown that growth substances accelerated digestion of starch in bean leaves (7, 8), and that indoleacetic acid caused starch to disappear from the endodermis of bean stems (2).

Material and methods

Indoleacetic or β-naphthoxyacetic acid was applied either in lanolin (1 per cent) to one side of cuttings or was added to the nutrient in which the cuttings were maintained or in which thin sections were placed. In the case of the lanolin treatments, observations were made after 6 days in the dark. The cuttings of these experiments were in a mineral nutrient which contained 1 per cent of either sucrose, glucose, levulose, maltose, or lactose.

Samples were preserved in Allen and Wilson's modification of Bouin's solution. Hand sections were cut and placed in 95 per cent alcohol. It is essential to remove the fixative thoroughly before testing for starch. The sections were then placed in an absolute alcoholic solution of iodine and cleared in xylol.

Experimental results

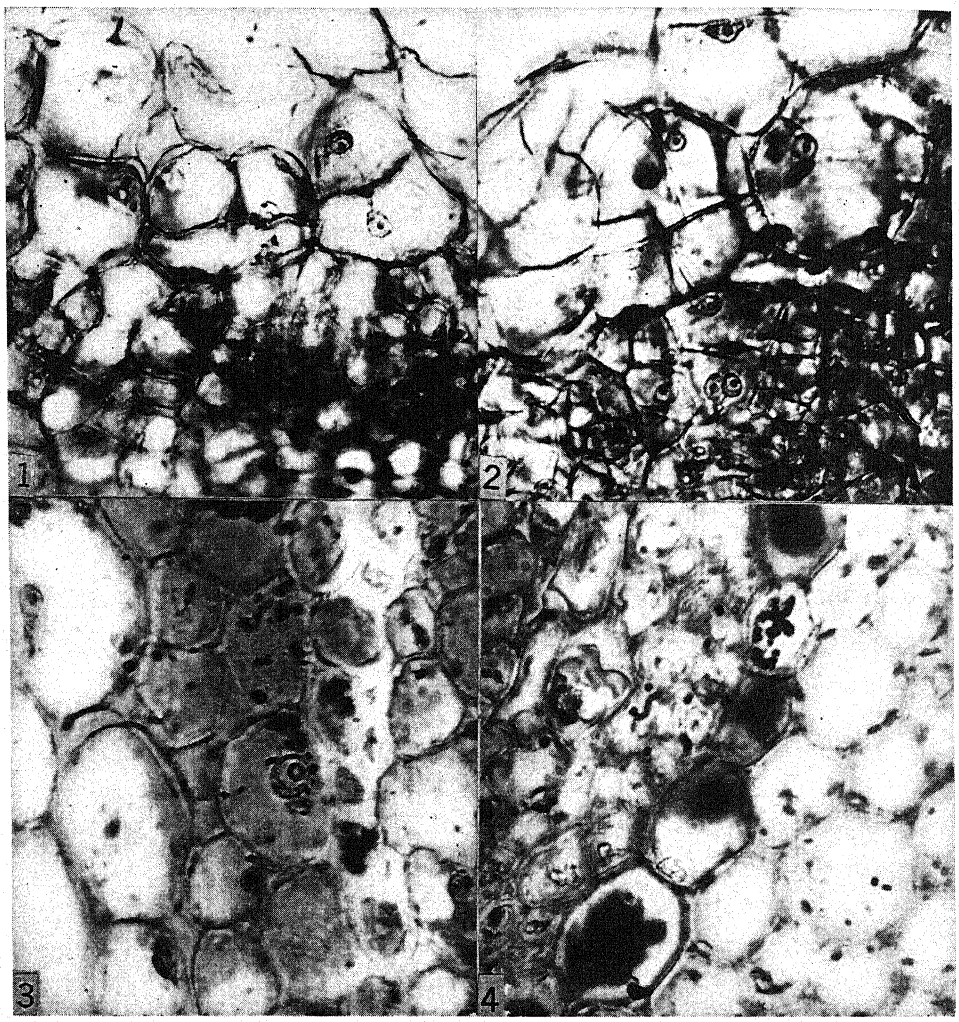
A large amount of starch is deposited in pith and endodermis of tomato cuttings cultured in the light in a sucrose medium. The plants kept in the dark for 6 days still have a copious supply, which is mostly confined to the endodermis. The same was true when sucrose was replaced by maltose, lactose, glucose, or even levulose—the only sugar used that was not convertible to glucose upon hydrolysis. Cuttings in the mineral nutrient alone were completely devoid of starch after 6 days in darkness.

Growth substance in lanolin

Both β-naphthoxyacetic and indoleacetic acid had a definite influence on the rate of hydrolysis of starch in tomato cuttings. Even in the presence of a high concentration of soluble carbohydrate the starch content decreased in the activated cells, although tardily. The endodermal cells might enlarge and divide and still retain a fairly large supply of starch. It was commonly present in the four daughter cells of the endodermis after two divisions (fig. 1). The grains, however, were much smaller in these cells than in that portion of the endodermis outside the influence of the growth substance, indicating that gradual solution was taking place. As activity increased the starch finally disappeared. The results were similar with all five of the sugars. Where the response was very strong, as cuttings in maltose, starch was to be found in the activated cells only where they bordered on the unaffected ones. In lactose cuttings, on the other hand, it was often present in the midst of the most active region.

Nitrogen-deficient tomato plants re-

acted in the same manner to the growth substances. A gradual diminution of the starch took place in the activated tissues, even in the light (fig. 2). On the contrary, starch was deposited in the endodermal derivatives which formed the root cap of the primordia. BORTHWICK, HAMNER, and PARKER (3) described the temporary storage of starch in very young primordia and its rapid disappearance therefrom. In the presence of a source of soluble carbohydrate, however, an abundance of starch might be found in the caps of young roots ready to emerge,



FIGS. 1–4.—Fig. 1, tomato cutting in 1 per cent levulose, treated with 1 per cent indoleacetic acid in lanolin; small starch grains still present in divided endodermis. Fig. 2, nitrogen-deficient tomato plant, treated with 1 per cent indoleacetic acid in lanolin; starch grains present in endodermis after two divisions. Fig. 3, bean cuttings in greenhouse 48 hours in sugar-free medium containing 0.02 per cent indoleacetic acid; starch grains small and less abundant. Fig. 4, as in fig. 3, but in 0.0002 per cent indoleacetic acid; starch grains large and abundant; effect similar to control.

while they were still under the influence of the growth substance. There was a sharp demarkation between the starch-storage cells of endodermal origin and the cells of the root proper.

The physiological peculiarity of the endodermis, which distinguishes it from other tissues in its facility to deposit starch, was retained by its direct lineal descendants. Here is a tissue retaining its special physiological function under morphologically different conditions. Starch was observed in the root cap in cuttings cultured in sucrose, maltose, glucose, and levulose. In the case of the last, a conversion to glucose very likely occurred (4). The root caps in nitrogen-deficient plants also contained starch.

Growth substance in solution in medium

1. Thin sections.—To test the effect of indoleacetic acid on the hydrolysis of starch by more direct contact, without the complicating factor of translocation, thin sections up to approximately 0.5 mm. were cultured in Petri dishes in nutrient solutions to which the growth substance was added. The results of trial experiments showed that a concentration of 0.02 per cent retarded the rate of hydrolysis of starch. Both bean and tomato were used. The tomato sections were of two types: those cut from plants growing in flats in the greenhouse and those from cuttings cultured in 1 per cent sucrose for 48 hours. The latter had a much higher starch content. The media, plus and minus sucrose, ranged in concentration of indoleacetic acid from 0.02, 0.002, 0.0002, 0.00002, to 0.000002 per cent. Controls contained no growth substance. The sections were preserved for study after 24 and 48 hours. Tomato sections were cut from the internode below the second expanded leaf from the apex; bean sections were cut from the lower portion of the third internode.

No quantitative differences could be noted, at least by the microchemical method, among any of the cultures containing sucrose. At the end of 48 hours starch was still abundant. Sugar retarded the hydrolysis of starch. In the minus-sucrose sections digestion of starch occurred rapidly in controls and concentrations of indoleacetic acid below 0.002 per cent, while those in 0.02 and 0.002 per cent contained abundant starch. The results were similar with the two tomato series and with the bean.

Thin sections in 0.02 per cent indoleacetic acid brought rapid decomposition of the growth substance. The solution became reddish brown after 24 hours, and the sections were stained a deep red.

Thus the starch present at the time of culturing, both in tomato and bean stems, was depleted faster in those cultures in which sugar was not present in the medium and faster in those with low concentrations of indoleacetic acid than in higher concentrations. The rate of hydrolysis in the lower concentrations was comparable with that of the controls in which indoleacetic acid was absent. The low concentrations of growth substance were apparently without effect. Under the conditions of these experiments, therefore, where indoleacetic acid was effective it acted in slackening the rate of hydrolysis of starch. Lindner (5) found that indoleacetic acid had no effect upon small segments of horseradish; he attributed this result to oxidizing enzymes or other unknown factors.

2. Cuttings.—To determine whether comparable tissues in the intact stem responded to aqueous solutions of the growth substance in the same way as the thin sections, kidney bean cuttings were maintained in similar series of indoleace-

tic-acid dilutions plus and minus sucrose, and controlled as indicated. One set of plants was put in the subdued light of the laboratory in the same place where the Petri dish cultures were kept; a second lot was returned to the greenhouse. In this way two levels of carbohydrate nutrition were established, one of which coincided with that of the thin sections. Fixations of the lower portion of the third internode were made after 24 and 48 hours.

Results with intact stems were different from those of the thin sections. The digestion of starch was definitely accelerated by 0.02 per cent indoleacetic acid.

Cuttings in laboratory.—Plants in the laboratory cultured in the sugar-free nutrient exhibited the clearest results. In 48 hours the controls and lower concentrations of indoleacetic acid still had considerable starch in endodermis and stomata but in no other tissue (fig. 7), while the 0.02 per cent cuttings were completely devoid of starch, except in the stomata. Moreover, marked cellular activity occurred. The endodermal cells enlarged and nuclei and nucleoli increased greatly in size in the cortex, endodermis, phloem parenchyma, cambium, and ray cells (fig. 5).

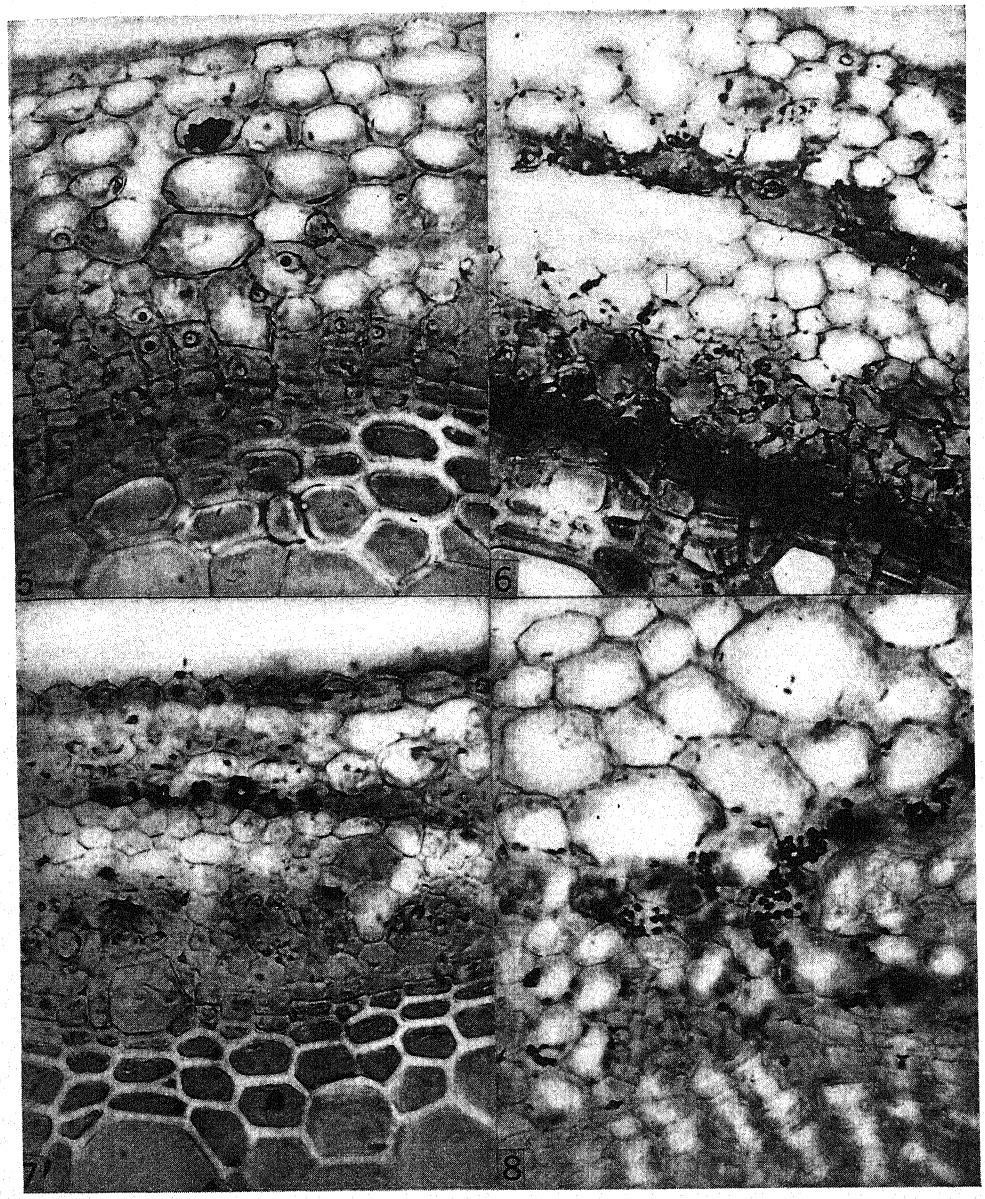
Cuttings in sucrose also showed after 48 hours a decrease in starch content when treated with 0.02 per cent indoleacetic acid. Only a small amount of starch remained in cortex, endodermis, and pith, but it was plentiful in the cambium (fig. 6). The controls and all greater dilutions of the growth substance had an obviously greater amount of starch in all tissues, with the exception of the cambium (fig. 8).

The effects were not very noticeable in 24 hours. In minus-sucrose cuttings 0.02 per cent indoleacetic acid hastened the hydrolysis of the starch in the endodermis only slightly more than in the controls, and the nucleoli of the activated tissues started to enlarge. In greater dilutions the starch was at least as plentiful as in the controls.

There were no noted differences in starch content in the plus-sucrose series after 1 day. The most obvious difference was the enlargement of nucleoli in endodermis, phloem, and cambium, especially with 0.02 per cent indoleacetic acid. Some nuclei with enlarged nucleoli were also noted in these tissues in 0.002 per cent dilution.

Cuttings in greenhouse.—The minus-sucrose cuttings cultured in the greenhouse in 0.02 per cent indoleacetic acid had some starch in endodermis and pith after 48 hours, but appreciably less than any of the other dilutions and less than the controls. The nuclei of endodermis, ray, phloem parenchyma, and cambium had enlarged greatly and contained large nucleoli (fig. 3). Large nucleoli were also present in plants treated with 0.002 per cent, but lower concentrations and controls did not show this effect (fig. 4).

Cuttings in the greenhouse, after 48 hours in a sucrose medium containing 0.02 per cent indoleacetic acid, had an abundance of starch in pith and endodermis. The amount, however, was less than in the controls. The amount of starch present in plants treated with 0.002 per cent was little different from that of the greater dilutions or the controls. In 0.02 and 0.002 per cent solutions nuclear enlargement occurred with corresponding increase in size of nucleoli, while higher dilutions and controls had small nuclei and nucleoli. In 24 hours these plus-sucrose cuttings did not differ much from the controls as to starch content or nuclear size.



FIGS. 5–8.—Fig. 5, bean cutting cultured 48 hours in 0.02 per cent indoleacetic acid in sugar-free medium in laboratory; starch has disappeared; nuclei and nucleoli have enlarged. Fig. 6, bean cutting in laboratory 48 hours in medium containing 0.02 per cent indoleacetic acid and 1 per cent sucrose; starch present in endodermis, phloem parenchyma, and especially in cambium. Fig. 7, as in fig. 5, but in 0.000002 per cent indoleacetic acid; starch still plentiful in endodermis; nuclei and nucleoli small; effect similar to control. Fig. 8, as in fig. 6, but without indoleacetic acid; much more starch in endodermis, less in cambium.

Gross responses accompanying depletion of starch

Bean cuttings in media containing 0.02 and 0.002 per cent indoleacetic acid, with and without sucrose, showed marked response within 1 hour after placing them in the solution. The leaflets apparently grew faster on the lower surface and curled symmetrically until their edges met. Upon further growth, one-half of the leaflet overlapped the other and they became rather tightly rolled and often twisted. Only the lower surface of the leaflet was now visible. The response was greater with 0.02 per cent and also with younger leaves. If left in 0.02 per cent indoleacetic acid more than 48 hours, wilting of the leaves occurred. The stem responded by curving, its final position in some cases being 90° from normal.

Link, Wilcox, and Link (6) described the upward curling of bean leaves treated locally on either upper or lower surfaces with indoleacetic acid in lanolin.

Discussion

The results of these experiments indicate that amylolysis follows treatment of tissues with a growth substance, even in the presence of high carbohydrate supply. Other effects accompany or precede the phenomenon, such as the gross responses in leaves and stems and cytological changes. For this reason it would be premature to assign a direct effect on the enzyme system. Nor is it possible to explain the inhibiting effect of indoleacetic acid on the digestion of starch in thin sections, in the present state of knowledge of the biochemical reactions of growth substances.

Stomatal starch was not noticeably affected by the growth substance. It persisted 48 hours after treating the minus-sucrose plants in the laboratory with 0.02 per cent indoleacetic acid, although starch completely disappeared from all other tissues. It also remained in all cultures of thin sections, even in controls, which became almost free of starch after 48 hours. Growth substances are not very effective in the epidermis, and the lack of influence on the hydrolysis of starch may be related to its general inactivity in this tissue.

Summary

1. Indoleacetic or β-naphthoxyacetic acid in lanolin brought about depletion of starch in tomato cuttings kept 6 days in darkness in a mineral nutrient containing sucrose. Similar cuttings, not treated, still had an abundance of starch after 6 days in darkness.

2. Cuttings in media containing maltose, levulose, dextrose, or lactose in place of sucrose responded in the same way to β-naphthoxyacetic or indoleacetic acid. Nitrogen-deficient plants also showed a depletion of starch when treated with the growth substances.

3. Starch was deposited in the root caps of primordia which resulted from treatment with the growth substance.

4. Digestion of starch was inhibited in thin sections by aqueous solutions of 0.02 or 0.002 per cent indoleacetic acid, while depletion of starch occurred rapidly in the controls. Lower concentrations of indoleacetic acid were ineffective. On the other hand, 0.02 per cent indoleacetic acid in aqueous solution accelerated the hydrolysis of starch in intact stems.

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LITERATURE CITED

1. BAUSOR, S. C., The interrelation of some organic materials in the growth substance response. BOT. GAZ. **103**:710–724. 1942.
2. BEAL, J. M., Effect of indoleacetic acid on thin sections and detached segments of the second internode of the bean. BOT. GAZ. **102**:366–372. 1940.
3. BORTHWICK, H. A., HAMNER, K. C., and PARKER, M. W., Histological and microchemical studies of the reactions of tomato plants to indoleacetic acid. BOT. GAZ. **98**:491–519. 1937.
4. LEONARD, O. A., Transformation of sugar in sugar beet and corn leaves and invertase activity. Amer. Jour. Bot. **25**:78–83. 1938.
5. LINDNER, R. C., Factors affecting regeneration of the horseradish root. Plant Physiol. **15**:161–181. 1940.
6. LINK, G. K. K., WILCOX, HAZEL W., and LINK, ADELINE D., Responses of bean and tomato to *Phytomonas tumefaciens* extracts, β-indoleacetic acid, and wounding. BOT. GAZ. **98**:816–867. 1937.
7. MITCHELL, J. W., KRAUS, E. J., and WHITEHEAD, MURIEL R., Starch hydrolysis in bean leaves following spraying with alpha naphthalene acetic acid emulsion. BOT. GAZ. **102**:97–104. 1940.
8. MITCHELL, J. W., and WHITEHEAD, MURIEL R., Starch hydrolysis in bean leaves as affected by application of growth-regulating substances. BOT. GAZ. **102**:393–399. 1940.

CHLOROPLAST SUBSTANCE OF SPINACH LEAVES[1]

C. L. COMAR[2]

Introduction

A detailed review of the structure of the chloroplast has been given by WEIER (17), who concludes: "The most critical observations indicate that the chloroplast consists of a colorless stroma or ground substance in which small discs of chlorophyll-impregnated cytoplasm are embedded. Additional evidence indicates that under conditions which are as yet imperfectly understood, the structure may vary from that noted above. It may appear perfectly homogeneous or striated." The small green particles, grana, which have been observed in normal chloroplasts, are considered to be the sole bearers of chlorophyll.

Various procedures are now available for the isolation of intact chloroplasts and chloroplast substance. The latter term is used after MENKE (11) to designate those chloroplast fractions in which the morphological structure of the chloroplast has been destroyed. When leaves are ground in water the green suspension contains nuclear and cytoplasmic material, cell wall fragments, intact and broken plastids, and grana. These components may be separated by fractional centrifugation (6, 9, 10, 12) or by a combination of centrifugation and selective flocculation (9, 13). The method of fractional centrifugation depends upon the fact that in the water extract the cell wall debris and unbroken cells, being heavier, are thrown down first, followed in order by larger crystals, starch grains, coagulated cytoplasm, chloroplasts, and grana.

The purpose of the work reported here was to study the chemical composition of chloroplast substance and thus to learn more of the medium which is of such importance in the photosynthetic process.

Experimentation

Fresh spinach leaves were used in all the following preparations.

PREPARATION 1.—This sample was prepared by fractional centrifugation as described by MOMMAERTS (12). Washed leaves were ground in distilled water to which a small amount of calcium carbonate was added. The crude press juice was centrifuged for 30 minutes at about 3700 r.p.m. The supernatant liquid was decanted and recentrifuged for 90 minutes at the same speed. The chloroplast substance was removed mechanically from the impurities, which appeared as a whitish zone in the lowest portion of the sediment, stirred in distilled water, and recentrifuged under the same conditions. This process was repeated twice.

PREPARATION 2.—The method of NEISH (13) was followed. The leaves were washed in distilled water, sliced with scissors, and ground in a porcelain mortar. The pulp was filtered through cheesecloth, and the filtrate centrifuged for about 10 minutes at 2000 r.p.m. The supernatant liquid was decanted into a 1-liter cylinder up to the 950-ml. mark, 50 ml. of $2M$ $CaCl_2$ added, and the solution mixed. After 30 minutes, flocculation had occurred and 800 ml. of the

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[2] Purdue Research Foundation Fellow 1938–40.

supernatant liquid was siphoned off. The remaining solution was centrifuged. The sediment was composed mainly of a white material covered with a thin layer of the green chloroplast substance. The white material was presumably flocculated cytoplasmic protein. The chloroplast substance was removed with a rubber policeman, triturated into distilled water, and recentrifuged. This process was repeated twice.

PREPARATION 3.—Freezing apparently decreased the solubility of the chloroplast substance. In solutions which had been frozen, this substance was almost completely thrown down by centrifugation in a few minutes at 3700 r.p.m.; ordinarily it takes 2–3 hours to throw down a small fraction at this speed.

This technique would facilitate the preparation and avoid the use of salts or other flocculating agents. However, it should first be demonstrated that the material thus obtained was at least as uncontaminated, particularly with regard to cytoplasmic proteins, as was that prepared by other methods. To determine this point three fractions were prepared:

Preparation 3A....Isolated by the usual method of centrifugation.
Preparation 3B....Isolated by centrifugation after storage at about −25° C. over-night.
Preparation 3C....Part of preparation 3B redispersed in distilled water and recentrifuged.

PREPARATIONS 5 AND 6.—These samples were prepared by fractional centrifugation. Details of procedure found to be convenient are as follows:

The leaves are washed thoroughly with distilled water and disintegrated in the Waring Blendor (5), in the presence of distilled water to which a small amount of calcium carbonate has been added; a minimum amount of water is used. The mixture is filtered through paper and the filtrate centrifuged for 30 minutes at about 3000 r.p.m. The residue is discarded and the supernatant liquid centrifuged at 3500–4000 r.p.m. for $2\frac{1}{2}$–3 hours. The chloroplast substance is separated mechanically from any impurities (which may be noted as a white zone in the sediment), dispersed in distilled water, and recentrifuged. This procedure is repeated if further impurities are observed.

METHODS OF ANALYSIS.—The sedimented chloroplast substance is dried under vacuum (20 μ) directly in the centrifuge tube and is kept ice-cold at all times. The dried material is powdered in an agate mortar and is then in a suitable form for chemical analysis.

LIPOID.—This term is used here to designate the fraction extracted by ether and alcohol-ether mixtures. The following method, based on that of MENKE (10), was developed and found to give reproducible results.

The apparatus consists of an alundum crucible suspended from the condenser of an all-glass Soxhlet extractor. The clean crucible is extracted with ether for 1–2 hours, dried under vacuum for about 12 hours, and weighed immediately on a micro-balance. (This process was repeated and the second weight obtained never differed from the first by more than 0.1 mg., which was sufficiently accurate since the samples averaged about 300 mg.) The sample is placed in the tared crucible, dried under vacuum, and the weight determined as before. The sample is extracted for 48 hours with freshly distilled ether, then for 48 hours with an alcohol-ether (3:1) mixture, and finally for 48 hours with ether again. The crucible is placed in vacuum for about 12 hours and then weighed in the usual manner.

NITROGEN.—Previous experiments indicated certain advantages in using the micro-Dumas rather than the micro-Kjeldahl method for the determination of nitrogen in the chloroplast substance. The apparatus was set up and the determinations performed after the method of NIEDERL and NIEDERL (14). Dry ice was used as a source of carbon dioxide and was found entirely satisfactory.

ASH.—The determination of ash was carried out on the lipoid-free material according to the method of PREGL (15), using a platinum crucible with lid.

CHLOROPHYLL.—Total chlorophyll and percentage component composition were determined spectroscopically (3, 4). In the case of preparation 5 the determination was made on the dry powdered material. In sample 6 the moist chloroplast substance was extracted directly after sedimentation, and the values corrected for moisture content.

Results

Table 1 presents the analytical values obtained for the various preparations. The results are calculated as percentages by weight of the dry chloroplast substance. Table 2 contains the nitrogen distribution of three preparations. Comparison with values obtained by other workers is found in table 3.

The chloroplast substance yielded a fairly large amount of acid-insoluble humin when hydrolyzed with hydrochloric acid, indicating the probable presence of carbohydrates and tryptophane. The following qualitative tests were performed on the chloroplast substance.

XANTHOPROTEIC.—This test was positive, indicating the presence of a phenyl group which may be easily nitrated. Tyrosine and tryptophane give this test readily, while phenylalanine does not usually give a positive reaction when nitric acid alone is used, as was the case here.

TABLE 1

COMPOSITION OF CHLOROPLAST SUBSTANCE FROM SPINACH LEAVES

PREPARATION NO.	PERCENTAGE				PROTEIN / LIPOID
	LIPOID	PROTEIN (% = N×6.25)	ASH	CHLOROPHYLL	
1	32.2	52.5	8.7	.....	1.63
2	22.5	53.8	7.3	.....	2.39
3A	30.3	55.3	6.2	.....	1.83
3B	27.5	56.8	4.6	.....	2.06
3C	27.9	60.7	2.7	.....	2.18
5	36.2	57.5	.....	5.27 (65.7%a)	1.59
6	36.7	52.2	.....	5.00 (67.6%a)	1.42

TABLE 2

NITROGEN DISTRIBUTION IN CHLOROPLAST SUBSTANCE

PREPARATION NO.	PERCENTAGE NITROGEN				LIPOID N IN CHLOROPLAST SUBSTANCE (GM. PER 100 GM.)
	UNEXTRACTED MATERIAL	EXTRACTED MATERIAL ON LIPOID-FREE BASIS	EXTRACTED MATERIAL CORRECTING FOR LIPOID CONTENT	TOTAL FOUND IN LIPOID FRACTION	
2	9.77	11.11	8.60	11.98	1.17
3A	9.92	12.69	8.85	11.06	1.07
3B	10.18	12.54	9.10	10.52	1.08

TABLE 3

PROTEIN-LIPOID RATIO OF CHLOROPLAST SUBSTANCE: COMPARISON WITH VALUES OF OTHER WORKERS

INVESTIGATOR	PLANT MATERIAL	PROTEIN / LIPOID
Menke (10)	Spinach	1.77
Chibnall (2)	Spinach	1.58
Neish (13)	Trifolium pratense	2.30
Comar	Spinach (preparations 1, 3A, 5, 6)	1.62
Comar	Spinach (preparations 2, 3B, 3C)	2.21

MILLON.—This test was positive, indicating the presence of tyrosine.

EHRLICH'S DIMETHYLAMINOBENZALDEHYDE.—This test was positive, indicating the presence of tryptophane.

LEAD ACETATE.—The presence of only a slight amount of loosely bound sulphur was indicated.

CARBOHYDRATE.—There was evidence that the chloroplast substance contained some carbohydrate material, but reducing activity and the presence of pentoses were not demonstrable, even after hydrolysis or disruption of the pigment-complex by organic solvents. The following tests were negative: Fehling's, Benedict's, picric acid, Bial's, and Seliwanoff's.

Discussion

MENKE (9) has shown that the cytoplasmic fraction of the typical green plant cell contains about 97 per cent protein and only a trace of lipoid. It follows, therefore, that contamination of the chloroplast substance with cytoplasmic material will cause the analysis of the former to show low lipoid and high protein values.

Suspensions of cytoplasmic substance, which is designated as a soluble protein fraction, are very labile, and GRANICK (7) noted that they may sometimes be flocculated by the presence of even minute quantities of neutral salts. This is substantiated by the high protein-lipoid ratio of preparation 2. It is also evident from preparations 3B and 3C that the cytoplasmic protein is sensitive to freezing. ANSON (1) has recently confirmed the decrease in solubility of chloroplast proteins as a result of exposure to freezing.

Some workers consider chloroplast substance, prepared by the methods outlined, to be identical with preparations of grana. This is not justifiable until it can be shown that the chloroplast preparation contains little or no cytoplasmic protein. It may also be noted that if these various preparations do represent the grana, they should have a higher chlorophyll content than have preparations of intact chloroplasts. MENKE (11) found that his preparations of intact chloroplasts had a higher chlorophyll and lipoid content than had his chloroplast substance. The work reported here can offer no information on this problem, since preparations of intact chloroplasts were not studied. The composition of the chloroplast substance has been characterized, however, and a parallel study on intact chloroplasts, using the preceding methods, should answer the question.

For purposes of comparison, the simple protein-lipoid ratio is used, since this avoids taking into account the varying amounts of ash and unidentified products present due to the different preparative procedures. Preparations 2, 3B, and 3C show high ratios, which indicate contamination with cytoplasmic proteins. Thus it appears that flocculation with calcium chloride or freezing should be avoided under the conditions used in these preparations.

The chloroplast substance contains about 54 per cent protein, assuming these proteins to contain 16 per cent nitrogen. The lipoid fraction contains about 11.2 per cent of the total nitrogen. About 30 per cent of the lipoid nitrogen is accounted for by chlorophyll; the remainder is possibly phosphatide nitrogen.

The agreement with other workers indicated in table 3 is close when it is remembered that NEISH used $CaCl_2$ as a flocculating agent. MENKE (10, 11) found 5.30 to 7.90 per cent chlorophyll, 56.4 per cent protein, and 31.9 per cent lipoid in his chloroplast substance isolated from spinach leaves. GRANICK (7), using preparations of intact chloroplasts from tomato leaves, found 13.2 per cent of the total nitrogen in the lipoid frac-

tion; he estimated 10 per cent to be chlorophyll nitrogen and the other 3.2 per cent to be due to phosphatides.

The possible stoichiometric relation between chlorophyll and protein has prompted many workers to obtain values for this ratio. Uncertainties in these values may be due to impurities in the chloroplast protein and errors in the determination of the chlorophyll. ZSCHEILE (18), after reviewing the literature to the middle of 1941, states, "It is the writer's opinion that none of the numerous chlorophyll analyses described in the literature can have sufficient accuracy to be more than very rough approximations, for one or both of two reasons (a) pure standards were not available for instrument calibration and (b) no account of *a* to *b* ratio variations could be taken in the method employed. Limitations of the physical technique employed have also been serious."

MOMMAERTS (12) found a value of about 5.5 parts of chlorophyll per 100 parts of protein. This low value was probably due chiefly to the fact that he assumed the ether-insoluble fraction of his preparation to be entirely protein. GRANICK'S (7) data indicated a value of about 27 parts of chlorophyll per 100 parts of protein. Here again the question of reference standards must be raised in the matter of the chlorophyll determination. MENKE (11) found a chlorophyll-protein ratio of 17.2 to 100 from analyses of preparations of intact chloroplasts from spinach leaves. He used a filter photometer with dried chlorophyll standards to estimate total chlorophyll.

SMITH (16) recorded a chlorophyll-protein ratio of 16.1 to 100 for chloroplast material prepared from leaves of *Spinacia* and *Aspidistra*. He purified the material either by high speed centrifugation or precipitation with salts. Chlorophyll was determined in aqueous digitonin solution by assuming a chlorophyll *a* to *b* ratio of 3 to 1 and using MACKINNEY'S (8) values of the extinction coefficients for the chlorophylls in acetone or ether solutions. Protein was estimated by the Kjeldahl method for nitrogen and corrected for the nitrogen present in the chlorophyll. Some downward revision of this value may result from consideration of the absorption coefficients of chlorophyll reported recently by ZSCHEILE and COMAR (19), which are significantly higher than those used by SMITH.

The data reported here indicate a ratio of 9 parts of chlorophyll per 100 parts protein in the chloroplast substance. MENKE (10) reported that chloroplast substance, having essentially the same chemical analysis as given here, contains about 15 per cent cytoplasmic substance. If this correction is applied, a value of about 12 parts of chlorophyll per 100 parts of protein is obtained for the chlorophyll protein complex.

Summary

1. Preparations of chloroplast substance isolated by fractional centrifugation showed less protein contamination than when flocculation with calcium chloride or freezing was used.

2. The chloroplast substance of spinach leaves has been found to contain about 54 per cent protein (N × 6.25), 34 per cent lipoid, 5 per cent chlorophyll, and 7 per cent ash. About 11 per cent of the total nitrogen is found in the lipoid fraction and less than one-third of this lipoid nitrogen is accounted for by chlorophyll.

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LITERATURE CITED

1. ANSON, M. L., On Lubimenko extracts of chlorophyll-protein. Science **93**:186–187. 1941.
2. CHIBNALL, A. C., Protein metabolism in the plant. Yale University Press, New Haven. 1939.
3. COMAR, C. L., and ZSCHEILE, F. P., Spectroscopic analysis of plant extracts for chorophylls *a* and *b*. Plant Physiol. **16**:651–653. 1941.
4. ———, and ———, Analysis of plant extracts for chlorophylls *a* and *b* by a spectro-photoelectric method. Plant Physiol. **17**:198–209. 1942.
5. DAVIS, W. B., A substitute for the laboratory food grinder. Ind. Eng. Chem., News edition **17**:752. 1939.
6. GRANICK, S., Quantitative isolation of chloroplasts from higher plants. Amer. Jour. Bot. **25**:558–561. 1938.
7. ———, Chloroplast nitrogen of some higher plants. Amer. Jour. Bot. **25**:561–567. 1938.
8. MACKINNEY, G., Criteria for purity of chlorophyll preparations. Jour. Biol. Chem. **132**:91–109. 1940.
9. MENKE, W., Untersuchungen der einzelnen Zellorgane in Spinatblättern auf Grund präparativ-chemischer Methodik. Zeitschr. Bot. **32**: 273–295. 1938.
10. ———, Untersuchungen über das Protoplasma grüner Pflanzenzellen. I. Isolierung von Chloroplasten aus Spinatblättern. Zeitschr. physiol. Chem. **257**:43–48. 1938.
11. ———, Untersuchungen über das Protoplasma grüner Pflanzenzellen. II. Der Chlorophyllgehalt der Chloroplasten aus Spinatblättern. Zeitschr. physiol. Chem. **263**:100–103. 1940.
12. MOMMAERTS, W. F. H. M., Some chemical properties of the plastid-granum. Proc. Kon. Akad. Wetensch. Amsterdam **41**:896–903. 1938.
13. NEISH, A. C., Studies on chloroplasts. Biochem. Jour. **33**:293–299. 1939.
14. NIEDERL, J. B., and NIEDERL, V., Micromethods of quantitative organic elementary analysis. Wiley and Sons. 1938.
15. PREGL, F., Quantitative organic microanalysis. 3d ed. Blakiston's. 1937.
16. SMITH, E. L., The chlorophyll-protein compound of the green leaf. Jour. Gen. Physiol. **24**:565–582. 1941.
17. WEIER, E., The structure of the chloroplast. Bot. Rev. **4**:497–530. 1938.
18. ZSCHEILE, F. P., Plastid pigments, with special reference to their physical and photochemical properties and to analytical methods. Bot. Rev. **7**:587–648. 1941.
19. ZSCHEILE, F. P., and COMAR, C. L., Influence of preparative procedure on the purity of chlorophyll components as shown by absorption spectra. BOT. GAZ. **102**:463–481. 1941.

PHYSIOLOGICAL STUDIES ON MOSSES. IV. REGENERATION IN PHYSCOMITRIUM TURBINATUM[1]

SAMUEL L. MEYER

(WITH SIX FIGURES)

Introduction

The generalization that both gametophytic and sporophytic generations in the life cycle of many mosses exhibit remarkable powers of regeneration of protonemata is now a well established fact. Earlier studies of regeneration in mosses were supplemented by the work of HEALD (8), who added important observations and at the same time surveyed the history of the subject prior to 1898. Subsequent investigations involving regeneration in European species of mosses have been made by many workers (1, 2, 3, 6, 7, 10–13, 17, 18, 19, 20, 22, 23–33). LA RUE (9) has noted that "little has been done with American species."

The purpose of this investigation was to determine the possibilities for regeneration in *Physcomitrium turbinatum* (Michx.) Brid., an American species of the Funariaceae. Comparisons are made between these results and those observed by HEALD (8) in *Funaria hygrometrica* Hedw., a closely related species.

Material and methods

Plants of *Physcomitrium turbinatum* were collected from pots in the greenhouse. Benecke's solution, as used by WETTSTEIN (24), served as the nutrient medium. Plant parts were submerged in the nutrient solution in Stender and Petri dishes, and Erlenmeyer flasks. Cultures were illuminated by the artificial light source previously described (14) and also in a northern daylight exposure supplemented by light from Mazda daylight bulbs. All tests were run for a period of 6 weeks. Occasional comparisons were made with plants grown from primary protonemata submerged in the nutrient solution.

Observations

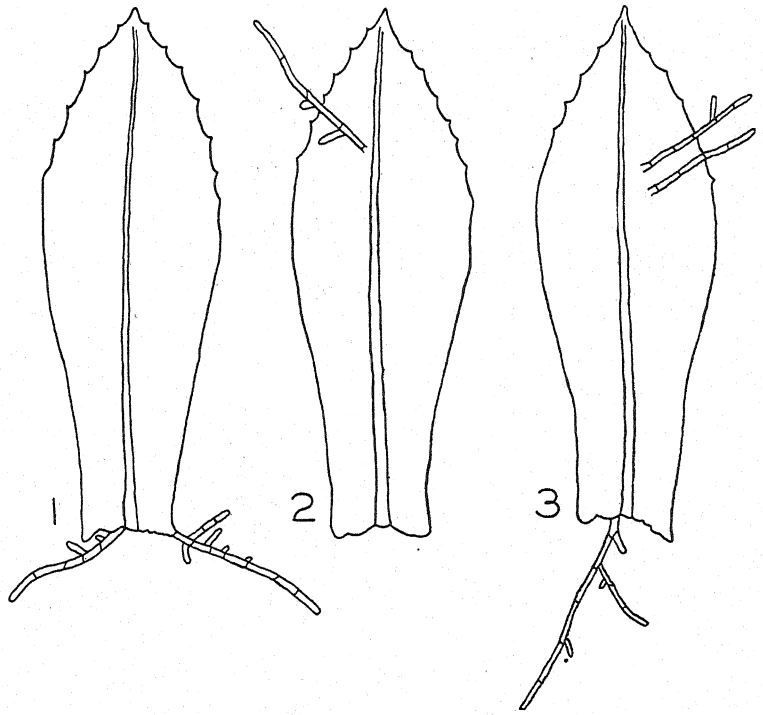
REGENERATION FROM GAMETOPHYTIC PARTS

Entire leaves were removed from stems and submerged in the nutrient solution. HEALD (8), who worked with *Funaria* only, stated that the leaves of that species were not "endowed with a very great power of regeneration," as "an average of about one out of six leaves showed signs of protonemata." In the closely related *Physcomitrium*, on the other hand, entire leaves regenerated readily. Of fifty leaves used, thirty-two developed protonemata. In only two cases did HEALD observe regeneration of protonemata from other than basal cells of the leaves of *Funaria*. In *Physcomitrium*, although eighteen of the entire leaves regenerated from basal cells alone (fig. 1), fourteen of the leaves showed protonemata developed from cells other than those at the base (figs. 2, 3). Of the latter, eleven leaves showed protonemata from the more distal portions (fig. 2), while three leaves showed both basal and distal regeneration (fig. 3).

[1] Contributions from the Botanical Laboratory, University of Tennessee, n. ser. no. 58.

In order to determine both the effect of a reduction in leaf area on the development of protonemata and whether the potentialities for regeneration are restricted to a particular portion of a leaf, detached leaves were divided into distal and proximal halves. Other leaves were divided into distal, middle, and proximal segments. There was no correlation between the segments which developed filaments and the portion of the leaf. Results clearly indicated that the tendency of detached leaves to regenerate is not confined to cells adjacent to the stem. Furthermore, reduction of the area of leaf segments to one-half or one-third had no well-defined influence on the regenerating activity of the cells.

Entire plants with leaves attached were submerged in the nutrient solution. No protonemata developed from the leaves during the experimental period of 6 weeks. Plants with attached leaves from which segments were removed were placed in the nutrient medium. No protonemata developed. These observations were comparable with those reported by HEALD for *Funaria*. It is of interest to compare this reaction of leaves of plants from the greenhouse with that of leaves of plants grown from primary protonemata submerged in the nutrient solution. Not infrequently protonemata developed from entire attached leaves of such water culture plants. In no case did filaments develop from attached leaves of plants grown in the greenhouse. These observations indicate that separation of leaves from stems is necessary to initiate regeneration of protonemata from leaves



FIGS. 1–3.—Leaves of *Physcomitrium turbinatum* illustrating basal (fig. 1), distal (fig. 2), and both basal and distal regeneration (fig. 3).

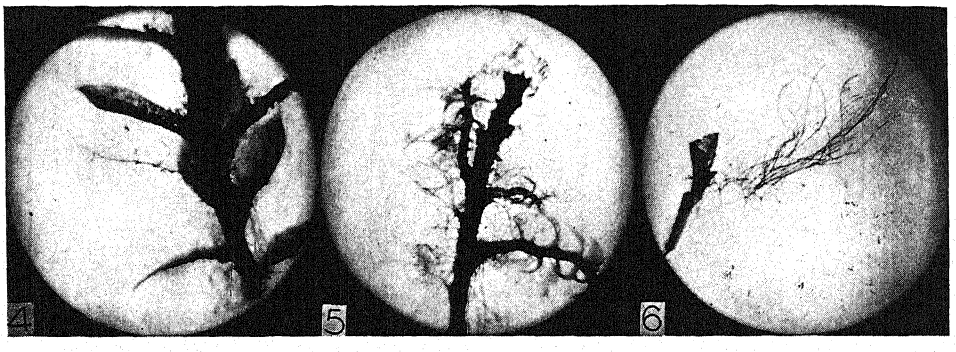
of aerial plants but is not necessary to initiate it from leaves of water culture plants.

Stems with leaves attached and those from which leaves were removed were placed in the nutrient medium. HEALD observed that intact plants of *Funaria* showed "no production of protonemata and only occasionally rhizoids." In *Physcomitrium*, both protonemata and rhizoids developed from stems of submerged uninjured plants (fig. 4) but not so abundantly as from defoliated stems (fig. 5). The latter were also characterized by the production of numerous branch shoots. In fact, this tendency of intact stems of *Physcomitrium* to produce filaments is also evident when the plants are grown in a humid atmosphere. Stems of leafy plants grown from protonemata submerged in the liquid medium and which have never come in direct contact with air also developed protonemata. This response indicates that the formation of filaments is not simply the reaction of cells grown in air to the presence of an aquatic stimulus but is actually part of the inherent potentialities of the cells, whether or not they have ever been subjected to an aerial environment.

Observations have been made on the development of protonemata from other gametophytic parts, such as archegonia, antheridia, paraphyses, and calyptrae. WETTSTEIN (24) has reported regeneration of all these structures in some species. As yet, sex organs, paraphyses, and intact calyptrae of *P. turbinatum* have shown no indications of regeneration of protonemata. One calyptra, injured by cutting, produced a filament (fig. 6).

REGENERATION FROM SPOROPHYTIC PARTS

The production of protonemata from sporophytic parts in artificial culture was first reported by PRINGSHEIM (15) for *Hypnum serpens*, *H. cupressiforme*, and *Bryum caespiticium*. Further consideration of this subject was presented by the same investigator later (16). STAHL (21) observed a similar reaction in *Ceratodon purpureus*, likewise in artificial culture. BRIZI (4) described from nature the production of protonemata by the sporophyte of *Funaria hygrometrica*. This tendency of sporophyte parts to regenerate has been of great value to those interested in the development of polyploid sexual plants.



FIGS. 4–6.—*Physcomitrium turbinatum:* Fig. 4, regeneration of protonemata from stem of submerged uninjured plant. Fig. 5, same from submerged defoliated stem. Fig. 6, same from calyptra, injured by cutting.

Setae of *Physcomitrium turbinatum*, of different degrees of maturity, were cut

just above the foot and below the capsule. They were then washed in distilled water, cut in shorter segments, and placed in Benecke's solution. Young green setae regenerated readily; those that were older, only infrequently. No filaments were formed from any setae that had lost their green color. In every case the filaments arose from the cut ends of the segments and never from epidermal cells. This observation has been recorded for other species of mosses, and it may be of some significance, since CAMPBELL (5) called attention to the fact that the cells of the setae which give rise to protonemal filaments "correspond in position to the sporogenous tissue of the capsule, and are probably homologous with them." STAHL (21), however, reported that the cells of the capsule wall gave rise to protonemata, in which case the suggested homology would not hold.

Summary

1. Detached leaves of *Physcomitrium turbinatum* give rise to protonemata when placed in a nutrient solution. Development of protonemata is not confined to any particular portion of the leaf.

2. Reduction in the amount of leaf tissue to one-half or one-third has no well-defined influence on the regenerating activity of the cells.

3. Attached leaves of greenhouse plants do not produce protonemata when submerged in a nutrient solution; those of water culture plants produce protonemata when submerged in a nutrient solution.

4. Defoliation of stems accelerates production of protonemata and branch shoots. Stems of both water culture and greenhouse plants react to an aquatic stimulus by developing protonemata.

5. A calyptra, injured by cutting, developed protonemata, although similar uninjured structures failed to regenerate.

6. Segments of young setae give rise to protonemata at their cut ends.

The greater portion of this investigation was carried on at the Miller School of Biology of the University of Virginia. The writer takes this opportunity of expressing appreciation to Professor IVEY F. LEWIS of that institution for helpful suggestions and valuable criticisms.

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LITERATURE CITED

1. BITTNER, K., Über Chlorophyllbildung im Finstern bei Kryptogamen. Öst. Bot. Zeitschr. **55**: 302–312. 1905.
2. BOAS, F., Zur Physiologie einiger Moose. Hedwigia **54**:14–21. 1913.
3. BORNHAGEN, H., Geschlechterverteilung und Geschlechtsdimorphismus bei *Splachnum ampullaceum* L. und *Splachnum sphaericum* (L. fil.) Swartz. Beih. Bot. Centralb. **46**:407–434. 1930.
4. BRIZI, U., Appunti di teratologia briologica del dott. Ann. R. Inst. Bot. Roma **5**:53–57. 1892.
5. CAMPBELL, D. H., The structure and development of mosses and ferns. New York. 1905.
6. CHALAUD, G., Nouvelles observations sur les rameaux foliares des Bryophytes. Rev. Gén. Bot. **39**:691–697. 1927.
7. CORRENS, C., Untersuchungen über die Vermehrung der Laubmoose durch Brutorgane und Stecklinge. Jena. 1899.
8. HEALD, F. D. F., A study of regeneration as exhibited by mosses. BOT. GAZ. **26**:169–210. 1898.
9. LA RUE, C. D., Regeneration in some American mosses. Papers Mich. Acad. Sci., Arts and Letters **11**:225–241. 1930.
10. MARCHAL, ÉL., and MARCHAL, ÉM., Recherches expérimentales sur la sexualité des spores chez

les Mousses dioïques. Mem. Cour. Acad. Belg. Cl. Sci. 1:1–50. 1906.

11. ———, and ———, Aposporie et sexualité chez les Mousses. Bull. Acad. Roy. Belg. Cl. Sci. 1907:765–789. 1907.
12. ———, and ———, Aposporie et sexualité chez les Mousses. II. Bull. Acad. Roy. Belg. Cl. Sci. 1909:1249–1288. 1909.
13. ———, and ———, Aposporie et sexualité chez les Mousses. III. Bull. Acad. Roy. Belg. Cl. Sci. 1911:750–778. 1911.
14. MEYER, S. L., Physiological studies on mosses. I. The development of leafy gametophytes in liquid media. Amer. Jour. Bot. 27:221–225. 1940.
15. PRINGSHEIM, N., Über vegetative Sprossung der Moosfrüchte. Monatsberichte der königlich preussischen Akad. Wissenschaften zu Berlin 1876:425–429. 1877.
16. ———, Ueber Sprossung der Moosfrüchte und den Generationswechsel der Thallophyten. Jahrb. wiss. Bot. 11:1–6. 1878.
17. SCHRATZ, E., Vergleichende Untersuchungen an uni- und bivalenten Laubmoosen. Biol. Zentralbl. 44:593–623. 1924.
18. SCHWEIZER, J., Polyploidie und Geschlechterverteilung bei *Splachnum sphaericum* (Linn. fil.) Swartz. Flora 116:1–72. 1923.
19. SERVETTAZ, C., Recherches expérimentales sur le développement et la nutrition des Mousses en milieux stérilisés. Ann. Sci. Nat. Bot. 17:111–223. 1913.
20. SPRINGER, E., Über apogame (vegetativ entstandene) Sporogone an der bivalenten Rasse des Laubmooses *Phascum cuspidatum*. Zeitschr. Indukt. Abstamm. Vererb. 69:249–262. 1935.
21. STAHL, E., Über künstlich hervorgerufene Protonemabildung an dem Sporogonium der Laubmoose. Bot. Zeitschr. 34:689–695. 1876.
22. WESTERDIJK, J., Zur Regeneration der Laubmoose. Rec. Travaux Bot. Néerlandais 3:1–66. 1907.
23. WETTSTEIN, F. VON, Kreuzungsversuche mit multiploiden Moosrassen. Biol. Zentralbl. 43: 71–83. 1923.
24. ———, Morphologie und Physiologie des Formwechsels der Moose auf genetischer Grundlage. I. Zeitschr. Indukt. Abstamm. Vererb. 33:1–236. 1924.
25. ———, Gattungskreuzungen bei Moosen. Zeitschr. Indukt. Abstamm. Vererb. 33:253–257. 1924.
26. ———, Kreuzengsversuche mit multiploiden Moosrassen. II. Biol. Zentralbl. 44:145–168. 1924.
27. ———, Genetische Untersuchungen an Moosen (Musci und Hepaticae). Bibliographia Genetica 1:1–38. 1925.
28. ———, Über plasmatische Vererbung, sowie Plasma- und Genwirkung. Nachr. Ges. Wiss. Göttingen Math.-Phys. Kl. 1926:250–281. 1927.
29. ———, Die Erscheinung der Heteroploidie, besonders im Pflanzenreich. Ergebn. Biol. 2:311–356. 1927.
30. ———, Morphologie und Physiologie des Formwechsels der Moose auf Genetischer Grundlage. II. Bibliotheca Genetica 10:1–216. 1928.
31. ———, Über plasmatische Vererbung und über Zusammenwirken von Genen und Plasma. Ber. deutsch. Bot. Ges. 46:(32)–(49). 1928.
32. ———, Über plasmatische Vererbung, sowie Plasma- und Genwirkung. II. Nachr. Ges. Wiss. Göttingen Math.-Phys. Kl. 1930:109–118. 1930.
33. ———, Bastardpolyploidie als Artbildungsvorgang bei Pflanzen. Naturwiss. 20:981–984. 1932.
34. WOESLER, A., Entwicklungsgeschichtliche und cytologische Untersuchungen an den Vorkeimen einiger Laubmoose, sowie einige Beobachtungen über die Entwicklung ihrer Stämmchen. Beiträge Biol. Pflanzen 21:59–116. 1933.
35. ———, Beitrag zur Kenntnis der vegetativen Vermehrung von *Sphagnum cymbifolium* Ehrh. Beiträge Biol. Pflanzen 22:13–24. 1934.

EFFECTS OF LIGHT QUALITY ON GROWTH AND MINERAL NUTRITION OF BEAN

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 546

LAWRENCE M. ROHRBAUGH

(WITH NINE FIGURES)

Introduction

The effect of light quality on the growth of plants has been reviewed by BURKHOLDER (1) and by POPP and BROWN (17). Various types of light sources and filters have been used, but in most cases the spectral regions incident to the plant were not known except as to their effect on the eye. This was especially true in the earlier experiments. Likewise the intensities of the light used have not been well controlled, and it is difficult to separate the effects of differing light quality from the effects of differing light quantity.

The use of fluorescent lamps as a source of light for growing plants has been reported by NAYLOR and GERNER (14), NAYLOR (13), and FLINT and MORELAND (4). Use of the different colors of fluorescent lamps which are available was suggested by NAYLOR (13) for the determination of some of the effects of light quality on plant growth. While most of these colored lamps emit some light in nearly all parts of the visible spectrum, the fact that most of the energy is emitted within comparatively narrow regions of the spectrum makes it possible to use filters with them which will absorb all the light from certain spectral regions without losing much of the radiated energy. Because of their efficiency in this respect, fluorescent lamps were chosen as a favorable source of light for the experiments on the effects of light quality reported in this paper.

Material and methods

LIGHT SOURCES AND FILTERS

Six 30-watt, 36-inch, fluorescent tubes were installed in metal reflectors as described by NAYLOR (13). Curtains made of double-thickness black sateen cloth were placed around each of the reflectors and extended almost to the floor. The inside of these curtains was painted white in order to increase and better equalize the light intensity at all points under the reflector. The flanges of the reflectors were fitted with sheet metal strips so that gelatin filters could be inserted in them to cover the entire lamp.

To eliminate certain parts of the spectrum, dyed gelatin filters were used. WHITMORE (27) has determined the transmission curves for many dyes, and three dyes were selected from this group which had regions of transmission and absorption suitable for these experiments and which would not fade under the lights. These were orange G, napthol yellow, and pontamine fast turquoise blue.

The gelatin filters were prepared in the following manner. Panes of window glass which had been cut to fit the reflectors (20×46 inches) were washed and dried. A pane was then placed on a table and carefully leveled with a spirit level. A gelatin mixture was prepared by heating about 75 gm. of clear gelatin and 30 gm. of sorbitol in 800 ml. of water on a steambath. This approximates 130 gm. of gelatin and 50 gm. of sorbitol per

square meter of surface covered. While this mixture was still warm, about 300 ml. of water containing the desired amount of dissolved dye was added and the entire mixture stirred. The amounts of gelatin, sorbitol, or water used are not critical. About 1100 ml. of the liquid worked very well in covering the 20×46 inches glass pane. The hot dyed gelatin mixture was cooled to about 60° C. and then poured on the glass plate. A glass rod was used to spread the gelatin to the sides and corners of the glass. The gelatin was filtered through several thicknesses of cheesecloth as it was poured in order to eliminate as many bubbles as possible. Any bubbles found on the poured filters were removed by touching them with the glass rod. While surface tension will prevent the melted gelatin from running over the edges of the glass if it does not become too deep and if strong currents are avoided, it was found that rubbing a piece of paraffin along the edges of the glass made accidents less frequent at this point. The filters should not be moved until thoroughly cooled. When dry, they were inserted in the reflectors, with the gelatin side down.

The orange G filter was prepared to contain 4 gm. of orange G (Dupont) per square meter. This filter transmitted no light of wave length shorter than 5300 A. The napthol yellow filter contained 5 gm. of napthol yellow (Coleman and Bell) per square meter and transmitted no light of wave length shorter than 4850 A. Two turquoise blue filters were used. One contained 4 gm. of pontamine fast turquoise blue (Dupont) per square meter and transmitted no light with wave length greater than 5750 A and only 0.5 per cent of the incident light at 5650 A. The other contained 2 gm. of the dye per square meter and transmitted some light of longer wave length. The transmission curves for these filters were determined with a Keuffel and Esser spectrophotometer (fig. 1). The quality of light reaching the plants is indicated by the spectrograms in figure 2. These spectrograms were made by using a small spectroscope with a camera. The film used was Eastman Panatomic X, and the time of exposure was equal in all cases.

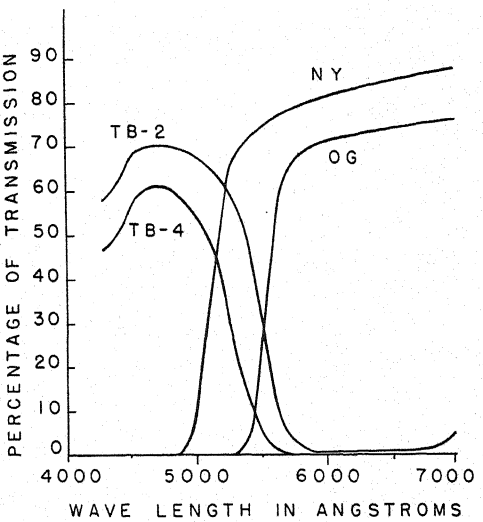


FIG. 1.—Transmission of light by dyed gelatin filters: TB-2, turquoise blue, 2 gm. per sq. m.; TB-4, turquoise blue, 4 gm. per sq. cm.; NY, napthol yellow, 5 gm. per sq. cm.; OG, orange G, 4 gm. per sq. cm.

The spectroscope was placed at varying distances from the different lamps, so that the incident energy at the spectroscope was equal for all the lamps. Because of the nature of the spectroscope, it is doubtful whether bands in the ultraviolet would have reached the film, even if they had been present in the incident light. Each of the lamps was fitted either with a gelatin filter or with a pane of window glass at the time the spectrograms were made. These spectrograms present a much more accurate measure of the quality of light emitted by the various colors of fluorescent tubes than is pre-

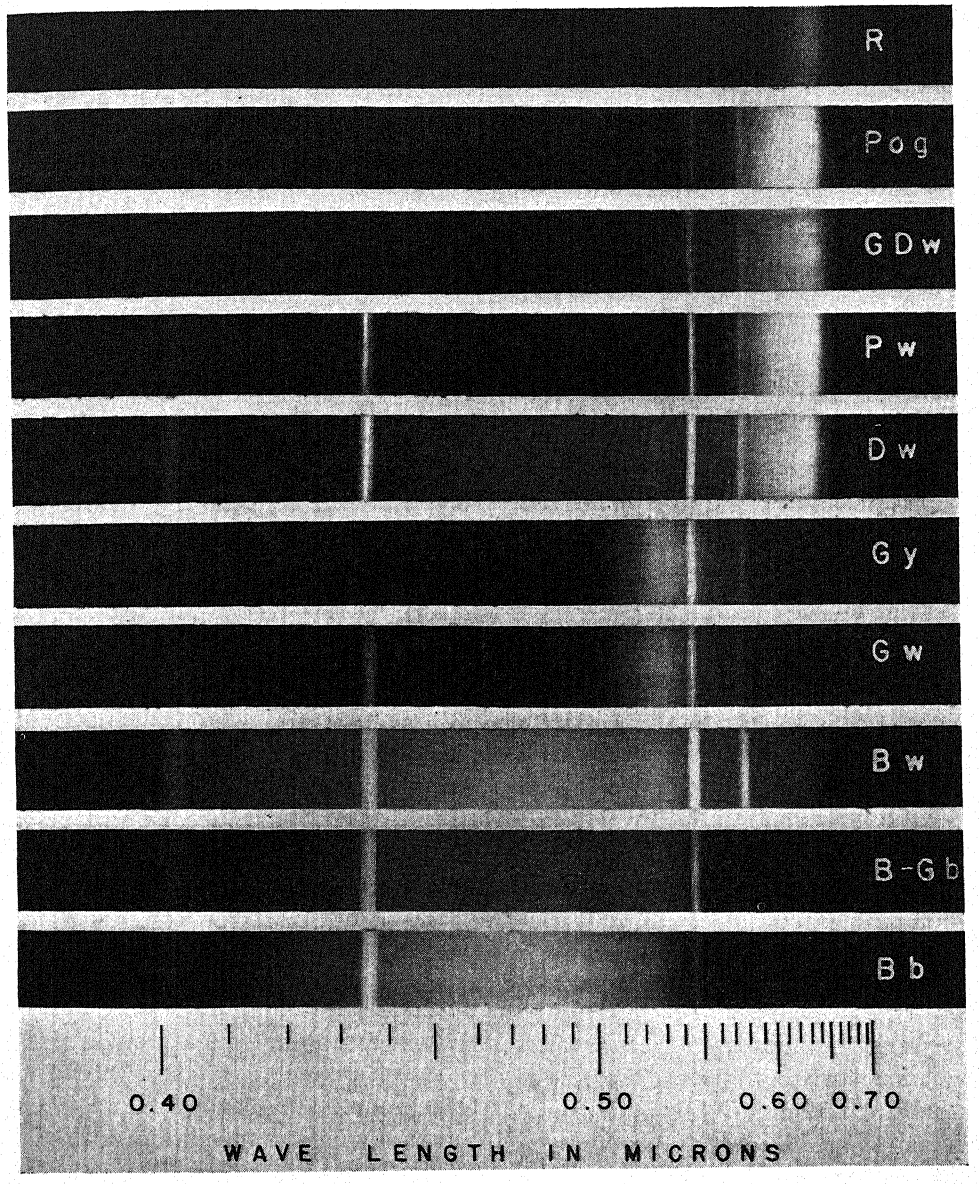


FIG. 2.—Spectrograms of light emitted by different colored fluorescent lamps with window glass and dyed gelatin filters: R, red without filter; Pog, pink with orange G filter; GDw, gold with window glass; Pw, pink with window glass; Dw, daylight (fluorescent) with window glass; Gy, green with napthol yellow filter; Gw, green with window glass; Bw, blue with window glass; B-Gb, blue and green with turquoise blue filter; Bb, blue with turquoise blue filter.

sented in the curves prepared by the manufacturer. Most of the energy is emitted in a series of very narrow bands of the spectrum and not in a broad band with gradual decreasing energy at either end, as represented by the manufacturer's curves.

MEASUREMENT OF RADIANT ENERGY

The total energy incident to a surface at the level of the plants was measured with a thermopile which had been calibrated with a standard lamp obtained from the United States Bureau of Standards,[1] and the distance from the lamps to the plants was varied so that the incident energy was approximately equal in all cases. The thermopile was connected with a balancing circuit, and a galvanometer was used as a null point indicator. A microammeter in the balancing circuit measured the amount of current needed to balance the thermopile current and provided a means of comparison with the standard lamp. The light intensities were also measured with a Weston footcandle meter. As the plants grew it became more difficult to maintain equal incident energy values, since the plants no longer presented a single plane of surfaces for the reception of light but rather a series of surfaces from the top of the plant down almost to the pot level. Equal energies at the pot level or at the top of the plant would be meaningless since the light sources were at widely varying distances. Therefore an attempt was made to estimate the average level of the plant surfaces, and the distances of the lamps were adjusted to produce equal incident energy at those levels.

[1] The thermopile was arranged and calibrated by Mr. R. A. WHITMORE, who also assisted in some of the measurements of light energy.

CULTURE OF PLANTS

Red kidney bean (*Phaseolus vulgaris*) plants were grown in quartz sand in glazed crocks. Five seeds were planted in each crock, and three rows of seven or eight crocks each were placed under each reflector. After development of the primary leaves, the plants were thinned to two uniform seedlings per crock. The positions of the three rows were changed each week in order to minimize the effects of different light intensities which may have existed between the center and outside positions.

Although the seeds were treated with dry Semesan before planting, many of the seedlings were lost because of infection with damping-off fungus. In the first two experiments the infected seedlings were replaced by transplanting ones of the same age which had been germinated in sand. In the later experiments, the crocks were kept in a darkroom in which the temperature was about 80° F. and the humidity low for several days after the seeds were planted. As soon as the first seedlings appeared above the surface of the sand, all the crocks were placed under the lights. This procedure apparently prevented activity of the fungus.

The plants were grown in a basement room, where the temperature was rather constant from day to day but varied from about 68° F. in winter to about 75° F. in summer. The temperature under the lamps was somewhat higher than this and not entirely the same under the different lamps. Thus on May 5 the temperatures under the various lamps were: pink with filter, 27.8° C.; gold, 28.9° C.; pink without filter, 27.0° C.; daylight, 26.8° C.; green with filter, 27.8° C.; green without filter, 27.8° C.; blue without filter, 27.1° C.; blue-green

with filter, 29.3° C.; and blue with filter, 28.4° C.

The plants were grown on cycles of 20 hours of light and 4 hours of dark in all the experiments. The composition of the nutrient solutions used is given in table 1. These solutions were applied as often as required to keep the top of the sand moist.

Measurements and chemical analysis

When harvested, the lengths of the hypocotyl and stem, the number of internodes, the number of flower buds, and the fresh weight of leaves and stems were determined. The roots were washed in salt water to free them of sand. The plants were dried at 80° C. in a forced-draft gas oven, and the dry weights of leaves, stems, and roots were determined. In later experiments the lengths of the first and second internodes and the average area of the leaflets were determined. The area of from thirty to eighty leaflets was measured by the photoelectric device described by Mitchell (12). No obviously juvenile leaves were included in these measurements.

The amounts of total ash, calcium, magnesium, potassium, and phosphorus were determined for the plants grown on complete nutrient solution in experiments 2 and 3. Semi-micro methods were used.

The dried tissue was ground in a micro-Wiley mill until it passed a 50-mesh screen. The powdered material was then dried to constant weight. Duplicate 0.5-gm. samples were weighed out and ashed in a muffle at 600° C. for 2 hours. After the weight of the ash had been determined, it was dissolved in 1:5 HCl, transferred to a 100-ml. volumetric flask, and made to volume with water. Aliquots were then taken for the determination of calcium, magnesium, potassium, and phosphorus.

Calcium was precipitated as the oxalate, as described by Kramer and Tisdall (9), and separated by centrifugation. Determination was made by titration with 0.01 normal potassium permanganate, using an electrometric titration assembly similar to that described by Kassner, Hunze, and Chatfield (8).

Magnesium was precipitated and separated from the supernatant liquid from the calcium determination according to the method of Kramer and Tisdall (9), as modified by Hammett and Adams (6), and was determined colorimetrically as described by Wall (23). The method of separation of calcium and magnesium

TABLE 1

Composition of nutrient solutions*

Nutrient solution	Amount of M/2 stock solution per liter of solution (ml.)					
	$Ca(NO_3)_2$	KH_2PO_4	NaH_2PO_4	KCl	$MgSO_4$	Na_2SO_4
Complete	12	3.0	.....	6.0	4.0	.....
Low K	12	1.0	2.0	.....	4.0	.....
Low P	12	0.025	.....	8.975	4.0	.....
Minus P	12	.....	.....	9.0	4.0	.....
Low Mg	12	3.0	.....	6.0	0.4	3.6
Minus Mg	12	3.0	.....	6.0	.....	4.0

* Micronutrients were also added to make the following concentrations: 0.5 p.p.m. of boron as $Na_2B_4O_7$, 0.5 p.p.m. of zinc as $ZnCl_2$, 0.5 p.p.m. of iron as ferric citrate, and 0.125 p.p.m. of copper as $CuCl_2$.

described by WALL was found to be satisfactory for the calcium, but it was not reliable for the small quantities of magnesium present in these tissues.

Potassium was precipitated as the cobaltinitrite and determination made colorimetrically on the basis of the color produced by the reaction of cobalt with Nitroso-R salt as described by SIDERIS (21).

Phosphorus was determined colorimetrically by the method described by WALL (23). All colorimetric determinations were made with a Keuffel and Esser spectrophotometer.

Experimentation

Red, gold, pink, daylight, green, and blue fluorescent tubes were used in experiment 1. Each reflector was fitted with different colored tubes. No window glass or filters were used under the reflectors in this experiment. The incident energy at the level of the plants was 2431 microwatts/sq. cm., or 581×10^{-6} gm. cal./sq. cm./sec. Measured in foot-candles, the intensity varied from 158 under the red to 460 under the gold.

The crocks under each reflector were divided into four groups, receiving complete, low magnesium, low potassium, and low phosphorus nutrient solutions. Because of the damping-off fungus which attacked the hypocotyls of the seedlings, most of the plants had to be replaced with ones transplanted from large germination crocks.

Eight reflectors were used in the second experiment. Two were fitted with pink tubes, two with green, two with blue, one with gold, and one with daylight. Under one set of the pink tubes was placed an orange G filter; under one set of the green tubes was placed a napthol yellow filter; and under one set of the blue tubes was placed a turquoise blue filter (4 gm. per sq. m.). Ordinary window glass was placed under all the other reflectors. The lamps were adjusted to give 1750 microwatts/sq. cm., or 418×10^{-6} gm. cal./sq. cm./sec., at the level of the plants. The intensity as measured with the Weston foot-candle meter varied from 270 foot-candles under the pink with filter to 360 foot-candles under the green with filter.

The damping-off fungus again necessitated using transplanted plants in most cases. The plants grown under each reflector were divided into three groups, one being watered with complete nutrient solution, one with low phosphorus solution, and one with minus phosphorus solution.

For experiment 3 the eight reflectors used in experiment 2 were again employed, and in addition a ninth reflector was used which was fitted with four blue tubes and two green tubes and a gelatin filter made with 2 gm. of turquoise blue per sq. m. The light intensities were adjusted as in experiment 2. Twenty-four crocks were placed under each reflector. These were divided into four groups of six crocks each. Three of these groups were given the three different levels of phosphorus nutrition used in experiment 2. The crocks of the fourth group were watered with complete nutrient solution, except for iron, which was supplied in amounts varying from none to 5 p.p.m.

In experiment 4 the nine reflectors of experiment 3 were again used with the same tubes and filters. The incident energy at the level of the plants in this experiment was 1314 microwatts/sq. cm., or 314×10^{-6} gm. cal./sq. cm./sec. Two-thirds of the plants were watered with complete nutrient solution and one-third with minus magnesium solution.

The same lamps and filters were again used in experiment 5. Some of the re-

flectors were repainted before beginning this experiment in order to raise the intensity of the light. The incident energy at the level of the plants was 1705 microwatts/sq. cm., or 408×10^{-6} gm. cal./sq. cm./sec. The plants were divided into two groups, which were watered with complete and minus magnesium nutrient solutions as in experiment 4.

Results

The plants grown in experiment 4 were harvested 31 days after planting, which was soon after the first flower buds became visible. In the other experiments the plants were harvested 39–51 days after planting, which was soon after flowering had started.

In all cases except experiment 4, visible flower buds appeared on plants grown under green light earlier than on plants grown under any of the other lamps. In experiment 4 flower buds appeared at approximately the same time on plants grown under all the lamps except the blue without the filter. Plants grown under this lamp did not have visible buds at the time of harvesting. Many of the flower buds which appeared on the plants grown under the green lights abscissed before the plants were harvested, so that the number of flower buds present at the time the plants were harvested was sometimes greater on the plants grown under the daylight and blue tubes without the filter.

The plants grown under the red tubes in experiment 1 were very unthrifty. They had short internodes and small and wrinkled leaflets. Since about one-half of these plants died before harvesting, it is possible that some other factor may have resulted in their poor growth, and comparison of these plants with those grown under the other lamps may not be valid.

Many of the plants grown under the gold, pink with filter, and green with filter became chlorotic toward the tops. Some of the younger leaves appeared entirely lacking in both chlorophyll and carotenoid pigments. This condition was not noticed in any of the plants which received energy from the blue end of the spectrum. The chlorosis was apparently unrelated to the amount of iron supplied to the plants, because it appeared equally severe in the plants given the different concentrations of iron in experiment 3.

Table 2 summarizes the data obtained from experiment 5. Some of the data for the other experiments are shown in figures 3–7.

The lengths of the hypocotyls and internodes of plants grown under the different lamps are shown in figure 3. The shortest hypocotyls and first internodes were found on plants grown under daylight and blue tubes without filters other than window glass. The spectrograms of figure 2 indicate that both of these lamps emit light from nearly all regions of the visible spectrum, but that the percentage of the total energy emitted in the orange-red region is very low in the blue tubes while rather high in the daylight tubes. The effect on the growth of the plants was much the same under the two lamps.

Plants grown under the blue tubes with the turquoise blue filter received no light with wave length longer than 5800 A and very little with wave length longer than 5600 A. Plants grown under this lamp produced longer hypocotyls and first internodes than were produced under any of the other lamps. The green tubes emit considerable energy in the blue line and some in the violet line of the mercury arc, but emit very little in the region above 6000 A. Plants grown under these tubes produced much shorter hypocotyls and first internodes than were

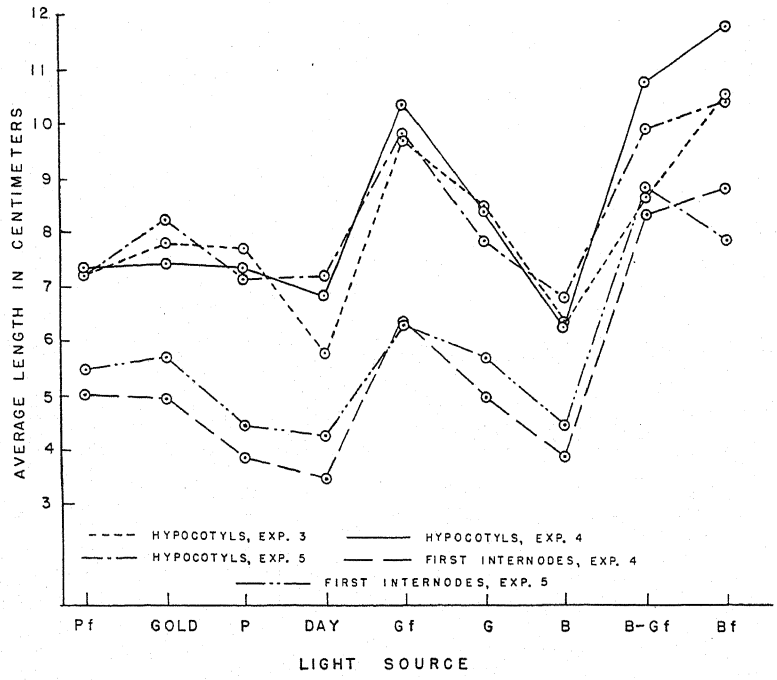


FIG. 3.—Average length of hypocotyls and first internodes of bean plants grown under different colored fluorescent lamps: Pf, pink with filter; Gold, gold without filter; P, pink without filter; Day, daylight (fluorescent) without filter; Gf, green with filter; G, green without filter; B, blue without filter; B-Gf, blue and green with filter; Bf, blue with filter.

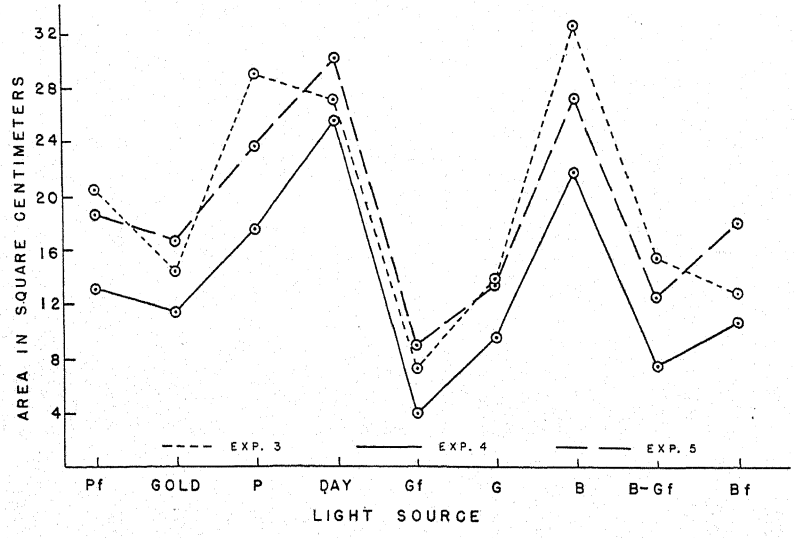


FIG. 4.—Average area of leaflets of bean plants grown under different colored fluorescent lamps: Pf, pink with filter; Gold, gold without filter; P, pink without filter; Day, daylight (fluorescent) without filter; Gf, green with filter; G, green without filter; B, blue without filter; B-Gf, blue and green with filter; Bf, blue with filter.

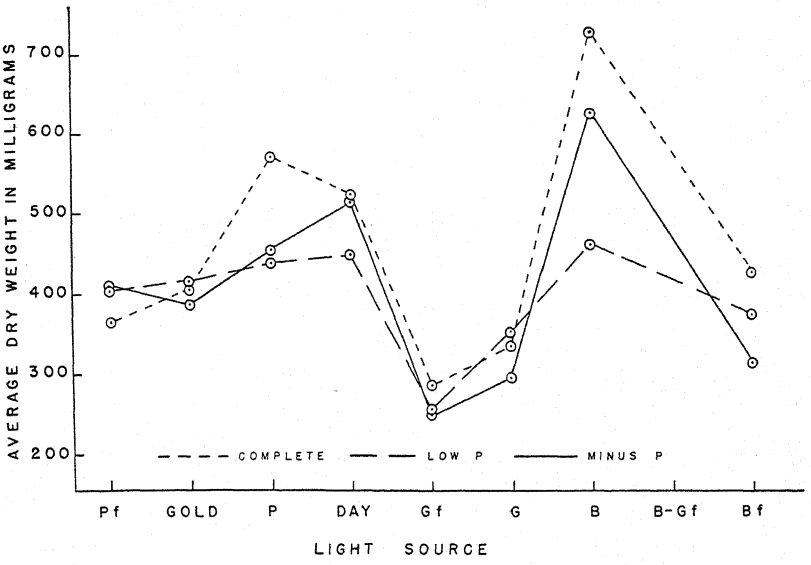


FIG. 5.—Average dry weights of bean plants grown on complete, low phosphorus, and minus phosphorus solutions under different colored fluorescent lamps; experiment 2: Pf, pink with filter; Gold, gold without filter; P, pink without filter; Day, daylight (fluorescent) without filter; Gf, green with filter; G, green without filter; B, blue without filter; B-Gf, blue and green with filter; Bf, blue with filter.

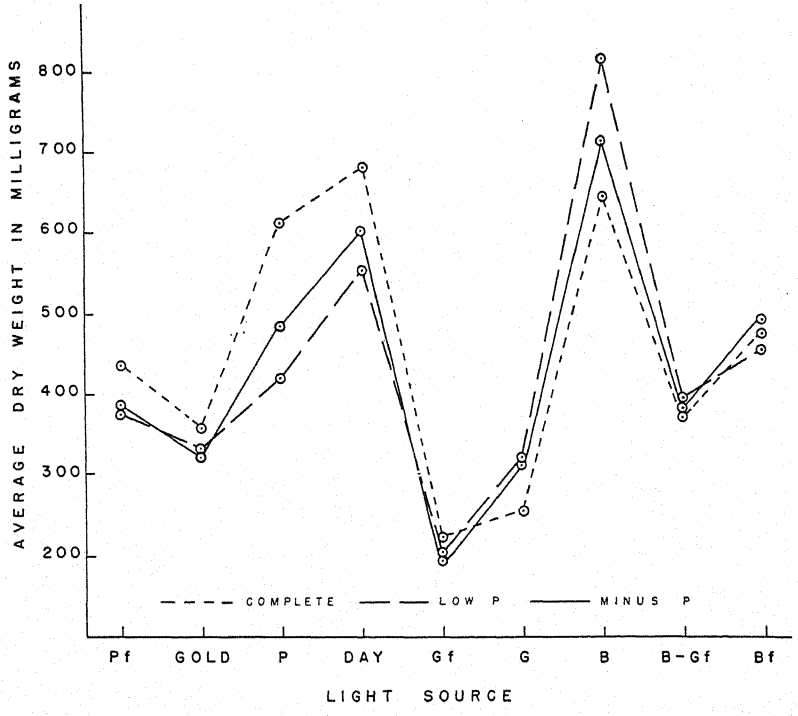


FIG. 6.—Average dry weights of bean plants grown on complete, low phosphorus, and minus phosphorus solutions under different colored fluorescent lamps; experiment 3: Pf, pink with filter; Gold, gold without filter; P, pink without filter; Day, daylight (fluorescent) without filter; Gf, green with filter; G, green without filter; B, blue without filter; B-Gf, blue and green with filter; Bf, blue with filter.

produced under the blue tubes with the filter but longer ones than were produced under the blue without the filter or under the gold, daylight, or pink tubes. In these cases red light was apparently much more effective in inhibiting growth in length of plants than was blue light. This was also indicated by the very short stems produced under the red tubes in experiment 1. The average length of the napthol yellow filter, the lengths of the hypocotyls and first internodes became almost as great as were obtained under the blue tubes with the filter. Also the hypocotyls and first internodes produced under the pink tubes with the orange G filter and under the gold tubes—neither of which emitted light of shorter wave length than about 4900 A and very little shorter than 5200 A—were longer than

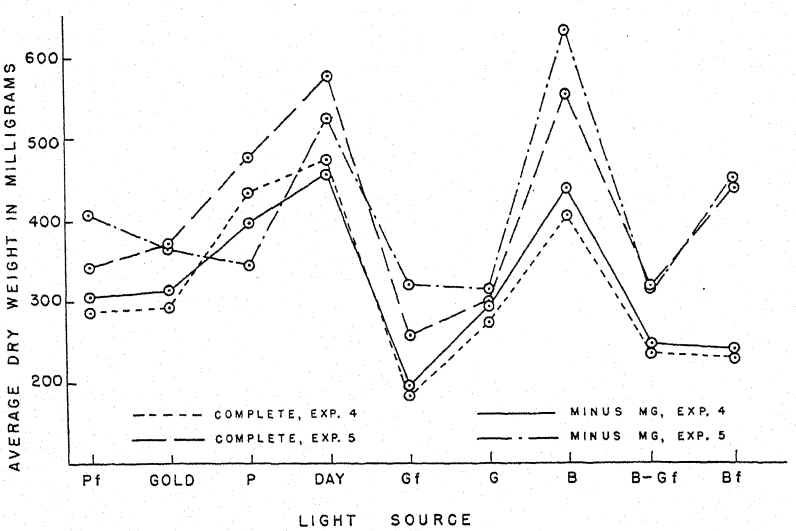


FIG. 7.—Average dry weights of bean plants grown on complete and minus magnesium solutions under different colored fluorescent lamps: Pf, pink with filter; Gold, gold without filter; P, pink without filter; Day, daylight (fluorescent) without filter; Gf, green with filter; G, green without filter; B, blue without filter; B-Gf, blue and green with filter; Bf, blue with filter.

internodes produced by these plants was 1.9 cm., while the average length of the internodes produced by the plants grown under the gold tubes in the same experiment was 4.3 cm., and plants grown under the other lights produced still longer internodes. About half the plants grown under the red tubes had died by the end of the experiment, however, and it is possible that some condition other than red light caused the short stems.

When the blue and violet light of the green tubes was absorbed by use of the those produced by plants subjected to the more balanced radiations of the daylight, blue, or pink tubes without filters. In these cases the blue end of the spectrum may have been somewhat effective in inhibiting stem elongation.

The lengths of the upper internodes did not seem to be affected by the different lamps in the same way as were the hypocotyls and lower internodes. Plants with long hypocotyls and first internodes tended to have shorter upper internodes than did those which had shorter hypocotyls and first internodes.

The average areas of the leaflets of the plants grown under the different lamps are shown in figure 4. The effect of the light quality on leaf area seems to be somewhat the opposite of the effect on elongation of stems. The largest leaves developed under the well-balanced light of the daylight and blue tubes without filters. The smallest developed under the green tubes with the filter. If only those lights are considered which emitted quite narrow regions of the spectrum (pink with filter, gold, green with filter, blue-green with filter, and blue with filter), then the largest leaves developed under the red end of the spectrum, the next largest under the blue end, and the smallest under the green region. The areas of the leaves of the plants grown under the red tubes in experiment 1 were not measured. They were probably smaller than those produced under any of the other lamps during that experiment. As already mentioned, it may not be safe to draw conclusions regarding the plants grown under the red tubes because of their very poor growth. But the leaf areas of the plants grown under the red tubes appear to be an exception to the general tendency for larger leaves under the red end of the spectrum.

TABLE 2

NUMBER OF FLOWER BUDS, AREA OF LEAFLETS, LENGTHS, WEIGHTS, AND TOP/ROOT RATIOS OF BEAN PLANTS GROWN WITH COMPLETE AND MINUS MAGNESIUM NUTRITION UNDER DIFFERENT CONDITIONS OF LIGHT QUALITY: EXPERIMENT 5

Light	Nutrient solution	No. of plants	Average no. of flower buds	Average area of leaflets (sq. cm.)	Average lengths					Average weights per plant					Top/root ratio
					Hypocotyls (cm.)	Stems (cm.)	First internodes (cm.)	Second internodes (cm.)	All internodes (cm.)	Leaves		Stems		Roots	
										Fresh (gm.)	Dry (gm.)	Fresh (gm.)	Dry (gm.)	Dry (gm.)	
Pf*..	Complete	28	0.18	18.7	7.29	36.1	5.55	5.70	6.10	2.21	0.166	1.46	0.117	0.062	4.56
	−Mg	13	0.23	.....	6.96	39.8	5.77	6.23	6.64	2.77	0.212	1.55	0.129	0.069	4.94
Gold.	Complete	25	0.80	16.9	8.30	41.6	5.80	5.96	6.89	2.44	0.182	1.56	0.128	0.064	4.84
	−Mg	15	0.47	.....	8.63	40.5	5.27	6.07	6.76	2.59	0.186	1.53	0.123	0.059	5.24
P....	Complete	25	0.44	23.9	7.20	42.1	4.54	5.60	7.11	3.29	0.260	1.67	0.146	0.072	5.64
	−Mg	15	0.20	.....	7.03	40.5	4.80	5.80	6.99	2.91	0.231	1.56	0.135	0.080	3.33
Day .	Complete	23	1.04	30.3	7.28	41.0	4.26	5.83	6.93	3.88	0.344	1.70	0.160	0.075	6.72
	−Mg	16	0.56	.....	7.44	39.3	3.84	5.37	6.69	3.44	0.290	1.76	0.159	0.076	5.91
Gf...	Complete	25	2.16	9.0	9.90	32.2	6.34	6.48	5.87	1.27	0.107	1.40	0.102	0.050	4.18
	−Mg	14	3.50	.....	9.57	33.4	6.75	6.96	6.24	1.66	0.141	1.49	0.112	0.067	3.78
G....	Complete	25	2.96	13.4	7.86	36.4	5.74	6.20	6.32	1.85	0.140	1.30	0.104	0.053	4.60
	−Mg	12	2.58	.....	8.17	35.5	5.54	5.96	6.09	1.86	0.143	1.33	0.106	0.063	3.95
B....	Complete	20	0.50	27.2	6.83	43.4	4.40	6.35	7.11	3.64	0.331	1.73	0.155	0.067	7.25
	−Mg	14	0.71	.....	6.46	42.9	4.61	6.68	7.23	4.38	0.385	1.74	0.164	0.082	6.70
B-Gf.	Complete	25	0.04	12.5	9.94	42.1	8.88	7.98	7.41	1.56	0.134	1.67	0.120	0.060	4.23
	−Mg	13	0.00	.....	9.96	38.8	8.50	7.73	7.11	1.52	0.131	1.66	0.122	0.058	4.36
Bf...	Complete	22	1.23	18.1	10.48	49.1	7.89	9.41	8.86	2.39	0.212	1.97	0.165	0.060	6.28
	−Mg	12	1.17	.....	10.21	50.1	7.92	8.88	8.71	2.37	0.218	2.00	0.163	0.068	5.60

* Pf, pink with filter; Gold, gold without filter; P, pink without filter; Day, daylight fluorescent without filter; Gf, green with filter; G, green without filter; B, blue without filter; B-Gf, blue-green with filter; and Bf, blue with filter.

No detectable deficiency symptoms developed in the plants grown on low or minus phosphorus or on minus magnesium solutions during these experiments. A comparison of the dry weights produced by the plants grown under the different conditions of nutrition and light quality is shown in figures 5–7. While in many cases the dry weight produced by

plants grown on complete nutrient solution was greater than that produced by plants grown on the solutions deficient in phosphorus or magnesium, in many cases the opposite condition was found. It is probable that the number of plants used was too small for any of these differences to be considered significant. Failure to obtain typical deficiency symptoms probably resulted from the storage of the necessary elements in the seed and from the low light intensities used.

Mineral analyses were made on the leaves and stems of the plants grown on complete nutrient solutions in experiments 2 and 3. The results of these analyses are shown in tables 3 and 4. While the amount of ash, expressed as percentage dry weight, did vary somewhat among plants grown under the different lights, the direction of variance was not the same in the two experiments in which mineral analyses were made. It is probable that these differences are of little significance.

No consistent differences were found in the amounts of potassium present under the different conditions of light quality, except that the highest values for the leaves and for the entire tops were found in plants grown under the green tubes without the filter, and the

TABLE 3

AMOUNT OF TOTAL ASH, CALCIUM, MAGNESIUM, POTASSIUM, AND PHOSPHORUS IN LEAVES, STEMS AND TOPS OF BEAN PLANTS GROWN UNDER DIFFERENT CONDITIONS OF LIGHT QUALITY: EXPERIMENT 2

Plant tissue	Light	Ash		Calcium		Magnesium		Potassium		Phosphorus	
		Percentage dry weight	Milligrams per plant	Percentage ash	Percentage dry weight	Percentage ash	Percentage dry weight	Percentage ash	Percentage dry weight	Percentage ash	Percentage dry weight
Stems	Pf*	12.8	13.1	15.8	2.03	2.03	0.26	38.2	4.89	5.19	0.67
	Gold	16.6	16.9	14.5	2.11	1.79	0.26	41.3	6.01	5.66	0.82
	P	13.6	19.8	12.1	1.64	1.09	0.15	42.0	5.70	5.04	0.68
	Day	12.2	16.5	13.1	1.60	2.11	0.26	42.1	5.15	4.99	0.61
	Gf	14.0	13.6	13.6	1.91	2.07	0.29	39.2	5.50	7.21	1.01
	G	13.8	12.8	13.2	1.81	1.85	0.25	44.3	6.10	5.67	0.78
	B	11.3	17.7	10.4	1.17	1.38	0.16	46.5	5.24	5.15	0.58
	Bf	12.0	15.2	11.0	1.32	2.41	0.29	43.5	5.22	5.90	0.71
Leaves	Pf	16.0	29.4	12.1	1.93	3.20	0.51	37.9	6.05	6.63	1.07
	Gold	17.5	34.9	12.5	2.18	2.75	0.48	41.0	7.16	6.90	1.21
	P	17.1	57.3	15.7	2.68	2.86	0.49	41.8	7.13	5.46	0.93
	Day	14.8	42.6	14.6	2.15	3.32	0.49	43.5	6.42	5.76	0.85
	Gf	16.7	20.8	12.7	2.12	3.30	0.55	41.8	6.97	6.84	1.14
	G	15.9	28.0	12.6	2.00	3.44	0.55	48.7	7.73	6.65	1.06
	B	14.8	65.3	15.7	2.32	3.51	0.52	41.1	6.06	5.30	0.78
	Bf	14.6	32.7	14.3	2.10	4.01	0.59	40.0	5.87	5.90	0.86
Tops	Pf	14.8	42.5	13.3	1.97	2.83	0.42	38.0	5.64	6.27	0.93
	Gold	16.4	51.8	13.2	2.16	2.25	0.37	41.1	6.74	6.52	1.07
	P	16.0	77.2	14.7	2.36	2.44	0.39	41.9	6.70	5.31	0.85
	Day	14.0	59.1	14.1	1.97	3.01	0.42	43.1	6.01	5.52	0.77
	Gf	15.5	34.5	13.0	2.02	2.77	0.43	41.6	6.30	6.96	1.08
	G	15.2	40.8	12.7	1.93	2.97	0.45	47.3	7.16	6.40	0.97
	B	13.8	83.0	14.6	2.02	3.11	0.43	42.3	5.85	5.27	0.73
	Bf	13.7	47.9	13.3	1.82	3.51	0.48	41.3	5.64	5.90	0.81

* See footnote to table 2 for explanation of abbreviations.

highest values for the stems were found under the blue without the filter.

The amount of calcium in the leaves of plants grown under the different lamps (fig. 8) followed an order similar to that for dry weight production and leaf area and was greatest in those plants receiving energy from most of the visible spectrum and lower when more selected regions of the spectrum were used. Removal of the red end of the spectrum resulted in about the same degree of lowering of the calcium content as did removal of the blue or of both ends. In general, the effect of light quality on the calcium content of the stems seemed to be the opposite of the effect noted for the leaves. When the calcium content of the leaves was high the content of the stems was low, and vice versa. If only those lamps are considered which emit light in rather narrow regions of the spectrum (pink with filter, gold, green with filter, blue-green with filter, and blue with filter), there was a decrease in the calcium content of the stems proceeding from the red to the blue end of the visible spectrum. Since the leaves made up the

TABLE 4

AMOUNT OF TOTAL ASH, CALCIUM, MAGNESIUM, POTASSIUM, AND PHOSPHORUS IN LEAVES, STEMS AND TOPS OF BEAN PLANTS GROWN UNDER DIFFERENT CONDITIONS OF LIGHT QUALITY: EXPERIMENT 3

Plant tissue	Light	Ash		Calcium		Magnesium		Potassium		Phosphorus	
		Percentage dry weight	Milligrams per plant	Percentage ash	Percentage dry weight	Percentage ash	Percentage dry weight	Percentage ash	Percentage dry weight	Percentage ash	Percentage dry weight
Stems	Pf*	14.0	18.1	14.2	1.99	1.70	0.24	41.2	5.77	5.28	0.74
	Gold	14.7	16.3	13.2	1.94	1.85	0.27	43.6	6.41	5.34	0.79
	P	15.1	26.4	11.2	1.68	1.06	0.16	36.0	5.42	4.07	0.61
	Day	14.3	24.7	11.1	1.58	1.13	0.16	43.1	6.16	4.08	0.58
	Gf	12.6	11.2	12.3	1.54	2.82	0.35	35.4	4.45	8.13	1.02
	G	14.6	13.6	11.8	1.72	1.99	0.29	42.1	6.16	5.71	0.84
	B	14.3	22.4	9.9	1.41	1.02	0.15	44.9	6.40	4.32	0.62
	B-Gf	14.3	16.5	12.3	1.75	1.82	0.26	38.4	5.48	5.54	0.79
	Bf	14.7	21.2	11.6	1.70	1.84	0.27	41.4	6.08	5.30	0.78
Leaves	Pf	18.1	42.6	13.7	2.49	2.75	0.50	36.3	6.58	4.70	0.85
	Gold	17.8	32.4	14.3	2.54	2.91	0.52	36.4	6.48	4.89	0.87
	P	17.6	62.7	17.4	3.05	2.64	0.46	33.7	5.92	3.87	0.68
	Day	17.5	72.6	18.2	3.18	2.68	0.47	32.1	5.61	3.82	0.67
	Gf	17.4	14.5	11.8	2.06	3.37	0.59	36.3	6.32	5.98	1.04
	G	17.2	19.6	14.4	2.48	3.19	0.55	37.1	6.38	5.26	0.90
	B	17.2	69.7	18.0	3.10	2.65	0.46	31.8	5.47	3.69	0.63
	B-Gf	17.9	35.0	15.5	2.76	2.85	0.51	33.8	6.04	4.29	0.77
	Bf	17.4	29.6	14.7	2.56	3.00	0.52	34.3	5.96	4.17	0.73
Tops	Pf	16.7	60.6	13.9	2.31	2.46	0.41	37.8	6.29	4.86	0.81
	Gold	16.6	48.7	13.9	2.31	2.59	0.43	38.8	6.45	5.05	0.84
	P	16.7	89.1	15.5	2.60	2.15	0.36	34.4	5.76	3.94	0.66
	Day	16.6	97.3	16.4	2.71	2.30	0.38	34.9	5.77	3.93	0.65
	Gf	14.9	25.7	12.0	1.79	3.15	0.47	35.9	5.35	6.84	1.02
	G	16.0	33.2	13.3	2.14	2.68	0.43	39.2	6.28	5.42	0.87
	B	16.4	92.0	16.1	2.63	2.26	0.37	35.0	5.73	3.85	0.63
	B-Gf	16.5	51.6	14.4	2.38	2.54	0.42	35.4	5.84	4.60	0.76
	Bf	16.2	50.7	13.4	2.16	2.48	0.40	37.3	6.02	4.64	0.75

* See footnote to table 2 for explanation of abbreviations.

greater part of the weight of most of the plants, the percentage of calcium in the entire tops was affected more by the content of the leaves than by that of the stems. Therefore the percentage of calcium in the tops followed an order similar to that for the leaves, but the degree of variation was less.

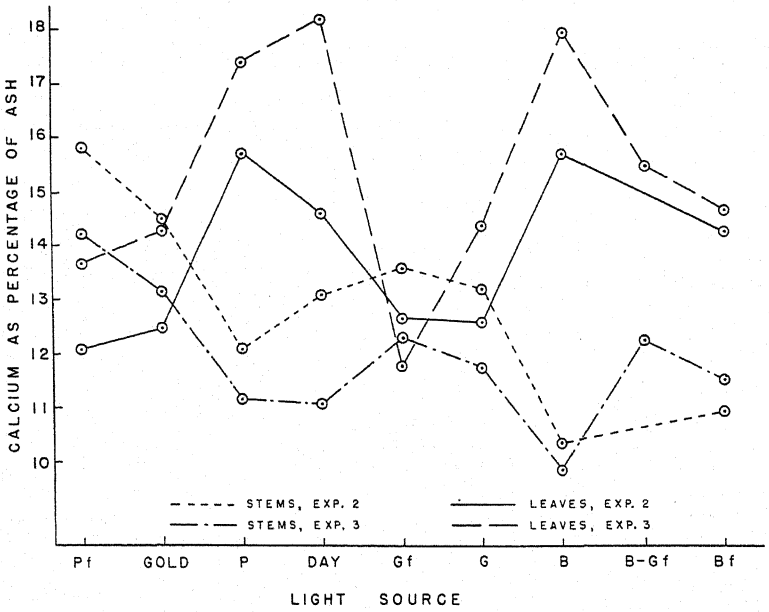


FIG. 8.—Calcium content of bean plants grown under different colored fluorescent lamps: Pf, pink with filter; Gold, gold without filter; P, pink without filter; Day, daylight (fluorescent) without filter; Gf, green with filter; G, green without filter; B, blue without filter; B-Gf, blue and green with filter; Bf, blue with filter.

The amount of phosphorus, expressed as percentage of ash, is shown in figure 9. It varied under the different lamps oppositely to the variation for calcium. The phosphorus content of those plants grown in the absence of light from the blue end of the spectrum and of those grown without light from the red end was higher than the phosphorus content of plants receiving the more balanced radiation. The phosphorus content was still higher when light from both ends of the spectrum was absent. In general, the amount of phosphorus expressed as percentage dry weight varied under the different lamps in the same way as did the amount expressed as percentage of ash.

There was little difference in the amount of magnesium, expressed as percentage of ash, in plants grown under the different lamps. Expressed as percentage dry weight, the amount of magnesium in the stems varied in somewhat the same way as did phosphorus. It was highest in plants grown under the green tubes with the filter and lowest in those grown under the pink, daylight, and blue tubes without filters. The variation in the leaves was less marked.

Discussion

There is considerable confusion in the literature regarding the effects of light quality on plant growth. Nearly all the earlier results indicated that the blue end of the spectrum inhibited stem elongation and produced a plant more or

less similar in appearance to those grown in daylight, while plants grown under the red end of the spectrum were long and slender. Most of the experiments were performed with colored glass or colored solutions as filters, and the quality of the light transmitted was not definitely known. Usually no attempt was made to equalize the intensities under the different filters.

same spectral glasses as employed by POPP but included another which transmitted the blue end and absorbed the red end. He used approximately equal intensities under the different filters, measuring them with thermopiles and with a Macbeth illuminometer. *Galinsoga*, sunflower, and *Geum* plants were grown under these filters. Plants grown under the blue filter were somewhat stunted,

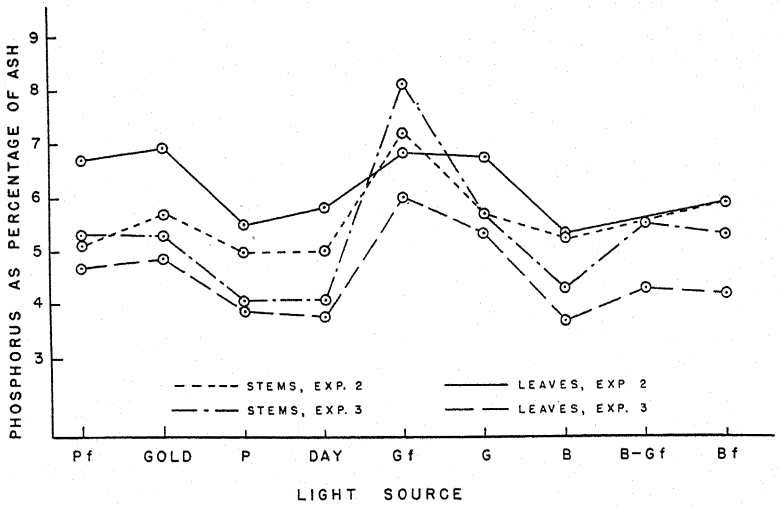


FIG. 9.—Phosphorus content of bean plants grown under different colored fluorescent lamps: Pf, pink with filter; Gold, gold without filter; P, pink without filter; Day, daylight (fluorescent) without filter; Gf, green with filter; G, green without filter; B, blue without filter; B-Gf, blue and green with filter; Bf, blue with filter.

More recently POPP (16), SHIRLEY (20), TEODORESCO (22), and others have reported similar results from experiments which were better controlled, although the light intensities were not fully equated in the experiments reported by POPP and were probably only approximate in many of those of TEODORESCO. POPP used various spectral glasses manufactured by the Corning Glass Company. When progressively greater regions of the blue end of the spectrum were removed, he obtained plants greater in length but containing less dry matter. SHIRLEY (20) used the having very short internodes but rather sturdy stems. Removal of the blue end of the spectrum resulted in very weak plants, with low dry weight production. Both POPP and SHIRLEY reported that the entire spectrum produced better plants than when any part of the spectrum was removed.

The results of the present experiments agree in part with the results obtained by POPP and by SHIRLEY. Plants getting light from all parts of the visible spectrum had the most normal appearance and produced greater dry weight than did those grown under lamps or filters

which removed part of the spectrum. It was also found that removal of the blue end resulted in plants which had somewhat longer hypocotyls and internodes and which produced less dry weight than did those receiving light from all parts of the spectrum. Curling and sometimes yellowing of the leaves grown in the absence of blue light was reported by POPP and by SHIRLEY, and these conditions were also noted in the present experiments.

If the results obtained under the blue tubes with the filter and under the combination of blue and green tubes with the filter are considered, however, the results are distinctly different from those obtained by SHIRLEY under blue glass. They also differ from the conclusions of POPP, TEODORESCO, and others that blue light inhibits the elongation of stems. If only those lamps are considered which emit light from comparatively narrow regions of the spectrum (blue with filter, combination of blue and green with filter, green with filter, gold, pink with filter, and red without filter), there was a decrease in the length of plants in the order named—from blue to red. These results indicate that, for the visible spectrum, the effectiveness in inhibiting the elongation of stems increases from blue to red. Similar results have been reported by DANGEARD (3), JOHNSTON (7), WITHROW (28), WENT (26), WEINTRAUB and MCALISTER (24), and GOODWIN (5). DANGEARD worked with *Lepidium*, WITHROW with bean, WENT with pea, and JOHNSTON, WEINTRAUB, and MCALISTER, and GOODWIN with the first internodes (mesocotyls) of *Avena*. LANGE (10) and SCHNEIDER (19) have also found that elongation of the first internode of *Avena* is definitely inhibited by irradiation with red light.

GOODWIN (5), JOHNSTON (7), and WEINTRAUB and MCALISTER (24) all found that the effectiveness of radiation in inhibiting elongation of the first internode of *Avena* increased from the blue to the red end of the visible spectrum but rapidly decreased from about 6600 A or 6800 A to the infra-red. GOODWIN also found that the effectiveness at 3650 A was considerably greater than at 4358 A. The results of POPP and MCILVAINE (18) on turnip plants also indicate that the effectiveness in limiting elongation and in decreasing the amount of growth substance increased from the violet end of the visible spectrum to the region of about 2350 A in the ultra-violet. Similar results were obtained by BURKHOLDER and JOHNSTON (2).

It therefore seems probable that the effectiveness of radiation in inhibiting stem elongation may have at least two maxima, one in the ultra-violet and one in the red, and at least two minima, one in the blue region of the visible spectrum and the other in the long red and infra-red. There are some indications, however, that radiation from all parts of the visible spectrum may be even more effective than an equal amount of radiation in the red region.

MCILVAINE and POPP (11) found that turnip seedlings grown under a Mazda lamp with a red filter were shorter than those grown under the same lamp with a green or violet filter. Similar results were obtained with these three filters when daylight was used as the light source. In these cases equal energies were used, but they point out that, because of the considerable amounts of infra-red, the intensity of the radiation from the visible region of the spectrum may not have been equal. These results agree with the generalization just made by the writer, although their other results do not agree so well.

In the experiments of McIlvaine and Popp (11), plants were grown under G34 and G403ED filters made by the Corning Glass Company. Three sources of light were used: a Mazda lamp, a mercury vapor lamp in quartz, and the combined radiation of a Mazda lamp and a mercury vapor lamp in glass. With all three sources, the plants grown under the red end of the spectrum as limited by the G34 filter were taller than those grown under the blue end as limited by the G403ED filter. Equal amounts of energy were applied to the plants from each end of the spectrum. These workers give 5290 to 7200 A as the spectral range of the G34 filter and 3740 to 5850 A for the range of the G403ED filter. Apparently these values were intended to pertain only to transmission in the visible spectrum. This is indicated by their reference to "the differences of transmission in the infra-red region" of the red, green, and violet filters for which the upper limit of transmission was listed as 685 mμ. The spectrograms published by Shirley (20) indicate that the G403ED filter transmitted radiation from 3550 to 5850 A. The G34 filter is known to transmit a considerable amount of infra-red. It seems probable, therefore, that a high percentage of the energy reaching the plants under the G34 filter—when the Mazda lamp was used as the source—was infra-red, which is relatively ineffective in inhibiting stem elongation, and that much of the inhibition of elongation found under the G403ED filter—when the mercury vapor lamps were used in the light source—may have resulted from irradiation with wave lengths shorter than 4000 A.

As mentioned in the writer's experiments with bean, the lengths of the upper internodes did not follow the same order, as shown by the hypocotyls and first internodes. There was a tendency for plants with short hypocotyls and first internodes to produce long internodes toward the top of the plant, and for those plants with long hypocotyls and first internodes to produce short internodes toward the top of the plant. This "compensating effect" was noticed by Went (26) in the case of Alaska peas but not in the case of Little Marvel peas. The amount of food supply may well have been the controlling factor for the upper internodes, since for the most part the plants with the greatest dry weight tended to produce longer internodes at the top of the plants. The relation between food supply and effect of auxin has been discussed by Went (25).

In general, the effect of light quality on leaf area in these experiments was similar to those noted by Went (26) for pea plants, but the development of very small leaflets on the plants grown under the red fluorescent tubes does not appear to be in agreement with Went's observations.

Nemec and Gracanin (15) reported that the amount of potassium found in rye plants grown under red and violet glass and in daylight was greater than in plants grown under green glass. The results described in this paper for bean plants do not seem similar. In these experiments no consistent differences were found in the amount of potassium present under the different conditions of light quality, except that the highest values in percentage of ash for the leaves and for the entire tops were found in plants grown under the green tubes without the filter, and the highest values for the stems were found under the blue tubes without the filter. Nemec and Gracanin also reported that the amount of phosphorus in the plants grown under the different glasses decreased as follows:

green glass, violet glass, sunlight, and red glass. The phosphorus content of the bean plants grown under the fluorescent tubes was greatest under green and least under light which most nearly resembled white light.

Plants grown under the more balanced radiation of the pink, daylight, and blue tubes without filters were high in leaf area, dry weight, and calcium content but low in phosphorus content; plants grown under the green tubes with the filter were lowest in leaf area, dry weight, and calcium content but highest in phosphorus content. While it would be expected that plants which have small leaves might produce small amounts of dry weight, it is probable that the correlation between these values may be more directly related to light quality than on that basis alone.

Summary

1. Red kidney bean plants were grown under fluorescent lamps of various colors. Dyed gelatin filters were used with some of the lamps to narrow the spectral regions used. The plants under each of the lamps received equal amounts of incident energy as measured with a thermopile.

2. The lengths of the hypocotyls and first internodes were least in the plants receiving light from nearly all parts of the visible spectrum. Light from the daylight and blue tubes produced very little differences in the plants. If only the plants grown under lamps and filters which emitted light from rather narrow regions of the spectrum are considered, the effectiveness in inhibiting elongation was greatest in the red and least in the blue regions.

3. The average leaf area and the production of dry weight were greatest in the plants receiving the more balanced radiation. If only the plants which received light from rather narrow regions of the spectrum are considered, the average leaf area was least in the green and slightly greater in the red than in the blue. Dry weight production was also least in the green but very nearly equal in the red and blue regions.

4. Visible flower buds developed earlier under green light than under the other lamps, but many of these buds abscissed.

5. No significant differences in the amount of total ash, or in the percentage of potassium or magnesium in the ash, were found in the plants grown under the different lamps.

6. The amount of calcium in the leaves, expressed as percentage of ash or of dry weight, was closely correlated with dry weight production and was highest in those plants receiving energy from most of the visible spectrum and lower when narrower regions were used. When the calcium content of the leaves was high, the content of the stems was low, and vice versa.

7. The amounts of phosphorus found in plants grown under the more balanced light of the daylight, pink, and blue tubes without filters were almost equal. It was higher in those plants which received no radiation in the blue and also in those which had received no radiation from the red end of the spectrum. It was still higher when light from both ends was absent.

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LITERATURE CITED

1. BURKHOLDER, P. R., The role of light in the life of plants. Bot. Rev. **2**:1–52. 1936.
2. BURKHOLDER, P. R., and JOHNSTON, E. S., Inactivation of plant growth substances by light. Smithsonian Misc. Coll. **95**:No. 20. 1937.
3. DANGEARD, P. A., Recherches sur l'assimilation chlorophyllienne et les questions qui s'y rattachent. Le Botaniste **19**:344–356. 1927.
4. FLINT, L. H., and MORELAND, C. F., A comparison of the effects of green light and red light on the simple leaf development of intact and decapitated bean plants. Plant Physiol. **17**:No. 4. 1942.
5. GOODWIN, R. H., On the inhibition of the first internode of *Avena* by light. Amer. Jour. Bot. **28**:325–332. 1941.
6. HAMMETT, F. S., and ADAMS, E. T., A colorimetric method for the determination of small amounts of magnesium. Jour. Biol. Chem. **52**: 211–215. 1922.
7. JOHNSTON, E. S., Growth of *Avena* coleoptile and first internode in different wave length bands of the visible spectrum. Smithsonian Misc. Coll. **96**:No. 6. 1937.
8. KASSNER, J. L., HUNZE, R. B., and CHATFIELD, J. N., A new use of the vacuum tube in electrometric titrations. I. Polarization of platinum electrodes in oxidation and reduction reactions. Jour. Amer. Chem. Soc. **54**:2278–2284. 1932.
9. KRAMER, BENJAMIN, and TISDALL, F. F., The direct quantitative determination of sodium, potassium, calcium, and magnesium in small amounts of blood. Jour. Biol. Chem. **48**:223–232. 1921.
10. LANGE, S., Über den Einfluss weissen und roten Lichtes auf die Entwicklung des Mesokotyls bei Haferkeimlingen. Jahrb. wiss. Bot. **71**:1–25. 1929.
11. MCILVAINE, H. R. C., and POPP, H. W., Further studies on growth substances in relation to the mechanism of the action of radiation on plants. Jour. Agr. Res. **60**:207–215. 1940.
12. MITCHELL, J. W., Measurement of the area of attached and detached leaves. Science **83**:334–336. 1936.
13. NAYLOR, A. W., The use of fluorescent light in experimental work. Trans. Illinois Acad. Sci. **34**:82–84. 1941.
14. NAYLOR, A. W., and GERNER, GLADYS, Fluorescent lamps as a source of light for growing plants. BOT. GAZ. **101**:715. 1940.
15. NEMEC, A., and GRACANIN, M., Influence de la lumière sur l'absorption de l'acide phosphorique et du potassium par les plantes. Compt. Rend. Acad. Sci. (Paris) **82**:806–808. 1926.
16. POPP, H. W., A physiological study of the effect of various ranges of wave length on the growth of plants. Amer. Jour. Bot. **13**:706–736. 1926.
17. POPP, H. W., and BROWN, F., Effects of different regions of the visible spectrum upon seed plants. Biological effects of radiation. B. M. DUGGAR, editor. Vol. 2, pp. 763–790. New York and London. 1936.
18. POPP, H. W., and MCILVAINE, H. R. C., Growth substances in relation to the mechanism of the action of radiation on plants. Jour. Agr. Res. **55**:931–936. 1937.
19. SCHNEIDER, C. L., The effect of red light on growth of the *Avena* seedling with special reference to the first internode. Amer. Jour. Bot. **28**: 878–886. 1941.
20. SHIRLEY, H. L., The influence of light intensity and light quality upon the growth of plants. Amer. Jour. Bot. **16**:354–390. 1929.
21. SIDERIS, C. P., Colorimetric microdetermination of cobalt and potassium. Ind. Eng. Chem. Anal. Ed. **9**:145–147. 1937.
22. TEODORESCO, E. D., Observations sur la croissance des plantes aux lumière de diverses longueurs d'onde. Ann. Sci. Nat. Bot. 10me series. **11**:201–335. 1929.
23. WALL, M. E., Microdetermination of some constituents of plant ash. Plant Physiol. **15**:537–545. 1940.
24. WEINTRAUB, R. L., and MCALISTER, E. D., Developmental physiology of the grass seedling. I. Inhibition of the mesocotyl of *Avena sativa* by continuous exposure to light of low intensities. Smithsonian Misc. Coll. **101**:No. 17. 1942.
25. WENT, F. W., Coleoptile growth as affected by auxin, aging, and food. Proc. Kon. Akad. Wetenschap. Amsterdam **38**:752–767. 1935.
26. ———, Effects of light on stem and leaf growth. Amer. Jour. Bot. **28**:83–95. 1941.
27. WHITMORE, R. A., Unpublished data. University of Chicago. 1942.
28. WITHROW, R. B., Response of seedlings to various wave bands of low light intensity radiation. Plant Physiol. **16**:241–256. 1941.

DEVICE FOR MEASURING ENTRY OF WATER INTO ROOTS[1]

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AND P. E. SKALING[4]

(WITH ELEVEN FIGURES)

Introduction

Relatively few methods have been devised for quantitative studies of water intake by roots that can be used to determine the gradient of absorption. The potometric technique appears best suited to this purpose, and apparatuses designed to use that method have been described (9, 10, 7, 8, 3). These devices are based on the use of calibrated capillary tubes attached to water-tight chambers through which the experimental root is passed (3, 10); or the use of capillary tubes with holes bored in the flattened ends through which the root can be threaded and adjusted with a mechanical manipulator (7, 8, 9). In the latter case the operations are carried on in an experimental chamber in which the atmosphere can be maintained at the saturation point.

These devices are suitable for study of the terminal, unbranched portions of roots with a relatively uniform diameter. They are not well adapted for study of the absorption of water and mineral nutrients by large or tapered roots, or by older portions of the root above a point where lateral roots have emerged, since it is not possible to pass the root through a series of chambers or capillaries. Further, these potometers are not suitable for the study of absorption where the root system is to be grown in a nutrient solution of high osmotic concentration, as it would be difficult to maintain uniform conditions with small volumes of solution.

Description of apparatus

For quantitative studies of water and salt intake, the following requirements were regarded as essential: (*a*) that quantitative measurements of intake could be made at various levels on an intact root so as to determine the gradient of absorption; (*b*) that the potometric units be designed so that they could be applied without injury to older regions of roots, even above the point of divergence of laterals; and (*c*) that the equipment could be used to study the absorption of water and salts from solutions of various osmotic concentrations maintained at uniform levels and under controlled environmental conditions.

The present device was designed with these requirements in mind. Each potometric unit of the apparatus (fig. 1) consists of a brass holder (*A*); a rubber chamber (*B*); a calibrated capillary tube with an attached scale (*C*); a glass plug (*D*); and a set screw (*E*). The brass holder consists of two hinged arms which can be tightened by means of the set screw. A section of brass tubing about 15 mm. long, extending through the inner face to form a 2-mm. shoulder, is soldered

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to each arm of the hinge. The brass tubing has an inside diameter sufficient to accommodate the rubber chamber snugly. The chamber is made of anode latex surgical tubing and may be of several sizes. One that has been found suitable for small roots, up to 3 mm. in diameter, has a $\frac{1}{4}$-inch inside diameter and a $\frac{1}{16}$-inch wall. A section of this tubing, 8–10 cm. in length, is inserted through the collars in the brass holder and is cemented to them with De Khotinsky's cement to prevent slipping when the hinge is tightened. After the rubber chamber has been cemented in place, a slot extending halfway through the tubing is cut in the surface toward the set screw. A hole is then burned through the center of the tube at right angles to the plane of the hinge and intersecting the inner margins of the slot (fig. 1, *4*). The size of the hole is gauged to accommodate the root to be used and is made with a steel punch heated to dull-red heat. It is desirable to have several sets of chambers with holes of different calibers so as to be able to attach a series of potometers to a tapered root without undue adjustment of the set screw.

The capillary tubes are about 15 cm. in length, and an enlarged cylindrical portion is fused to one end so that it can be inserted firmly in the rubber chamber. Each tube is calibrated to the scale attached to it, so that quantitative determinations of the amount of water absorbed by the portion of the root inclosed in the rubber chamber can be made. The metal scale is fastened to the capillary tube by means of small rubber bands and can be removed easily for cleaning. The glass plug is made so that it will fit snugly in the open end of the rubber chamber. Each potometer is held in place by a thermometer clamp attached to the brass holder, and the free end of the capillary tube rests in a bracket that can be adjusted so that it is in a horizontal posi-

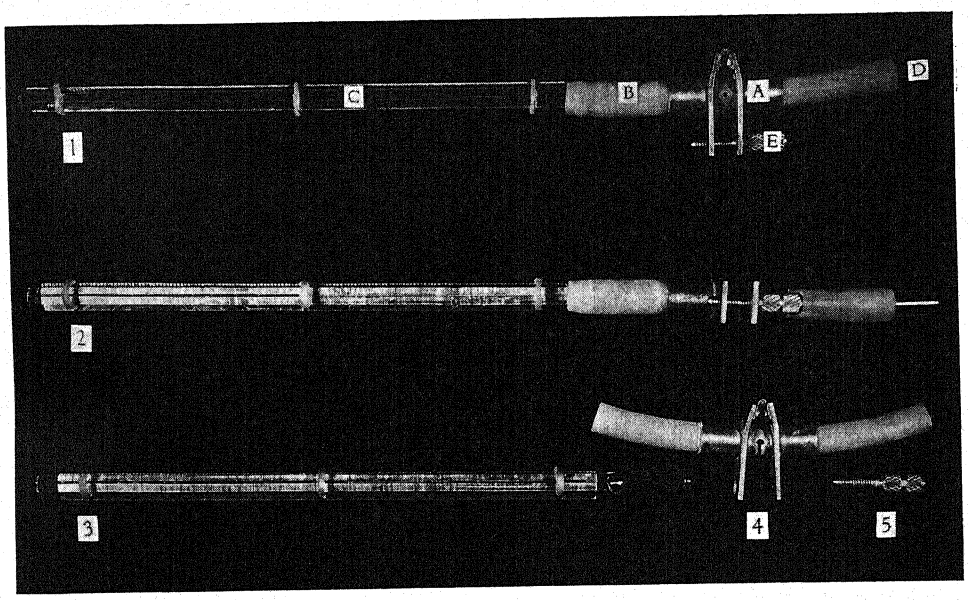


FIG. 1.—Potometric unit: *1* and *2*, two views of assembled potometer; *3*, *4*, and *5*, unassembled major parts. *A*, brass holder; *B*, rubber chamber; *C*, calibrated capillary tube and attached scale; *D*, glass plug; *E*, set screw.

tion. Several of the units can be attached to a single root at different levels and simultaneous readings of the water intake made to determine the gradient of water absorption (fig. 2).

The experimental plant and the attached potometers are held in place in an aquarium by clamps attached to a flexaframe rod. The aquarium is equipped with a carbon pipe aerator so that the

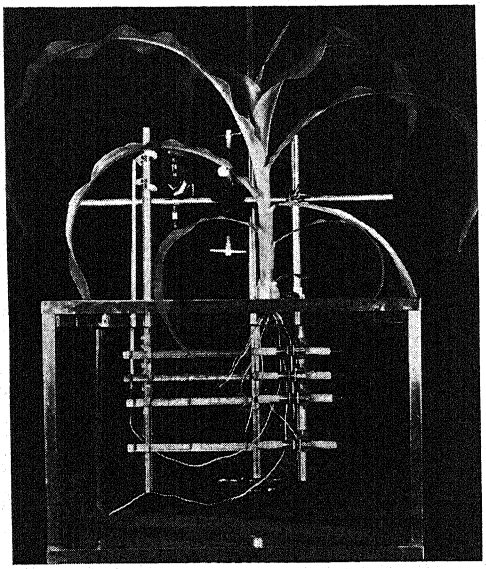


FIG. 2.—Final assembly, with corn plant. Potometers attached at three levels; the one at bottom is the check. One cycle of adventitious roots not excised.

roots can be subjected to varied oxygen supply. The apparatus is operated in a room in which the temperature and humidity can be maintained at relatively constant levels. This eliminates thermal variations, which would affect the operation of the potometers. Overhead illumination is supplied by Westinghouse fluorescent tubes.

Material and experimental procedure

ROSENE (7, 8) used the adventitious roots of the onion and excised all but a single root when the bulb was placed in the experimental chamber. This root has desirable features of uniformity in diameter and absence of lateral roots close to the apical meristem. BREWIG (1) and GREGORY and WOODFORD (3) used *Vicia faba*. HÖHN (4), experimenting with a different technique, used corn, wheat, and *Tradescantia*. In our experiments young corn plants, Mexican June variety, were used. These were grown in complete nutrient solution until 10–12 inches tall. At this stage they had about six expanded leaves and a cycle of unbranched adventitious roots 10–15 cm. in length.

The corn plant is supported in the aquarium so that the selected root can be fitted conveniently into the desired number of potometers (fig. 2). To avoid fracture or breakage of the root, it is desirable to have the potometric units carefully adjusted as to horizontal alignment and spacing before attempting to fit the root in the rubber chambers. By opening up the hinge, the root can be inserted through the slot in the rubber chamber and seated in the holes at the inner margin of the slot. The removable set screw is then inserted in the arms of the hinge and tightened gently. If the diameter of the root approximates that of the hole in the chamber, it is not necessary to tighten the set screw much in order to make the chamber air-tight. After the root is adjusted in the series of chambers, each one is tested for tightness by closing one end with the glass plug and applying a stream of air to the other opening in the chamber. The set screw is then slowly tightened until no bubbles escape around the root.

All air is then evacuated from the chamber by removing the glass plug and flushing the chamber with a stream of water. A rubber ear syringe is useful for

this purpose. After the air is completely evacuated, the capillary tube is inserted in one end of the chamber and adjusted in a horizontal position, and the other end is closed with the glass plug. The capillaries are set for the initial reading by drawing in an air bubble until it is on scale. This can be done by raising the assembled apparatus until the capillary is just below the surface of the water and then lifting the open end above the surface, at the same time exerting a slight pressure on the glass plug. When the pressure is released, a column of air is drawn into the capillary, the distance depending upon the initial pressure. The tube is then submerged and the air column shortened to a single bubble by manipulation of the glass plug. By starting at the lowest capillary in the series and setting the bubbles progressively toward the uppermost one, it is possible to adjust a set of four or five units without difficulty. In each assembly a check capillary is included to record any variations in movement that might be due to thermal effects. The check unit is set up in the same manner as the other potometers, except that a small glass tube is inserted through the holes in the rubber chamber in place of a root.

In most of the experiments with corn, all roots except the one tested were excised after the apparatus was completely assembled. This procedure resulted in greater uniformity in the quantitative results obtained as it tended to reduce the variation in intake due to differences in the total absorbing surface of the root systems. Results obtained when all the roots were left intact were similar with respect to gradient, however, although the rate of water intake per unit area of root surface was reduced. No roots were excised in experiments with older roots of *Citrus*.

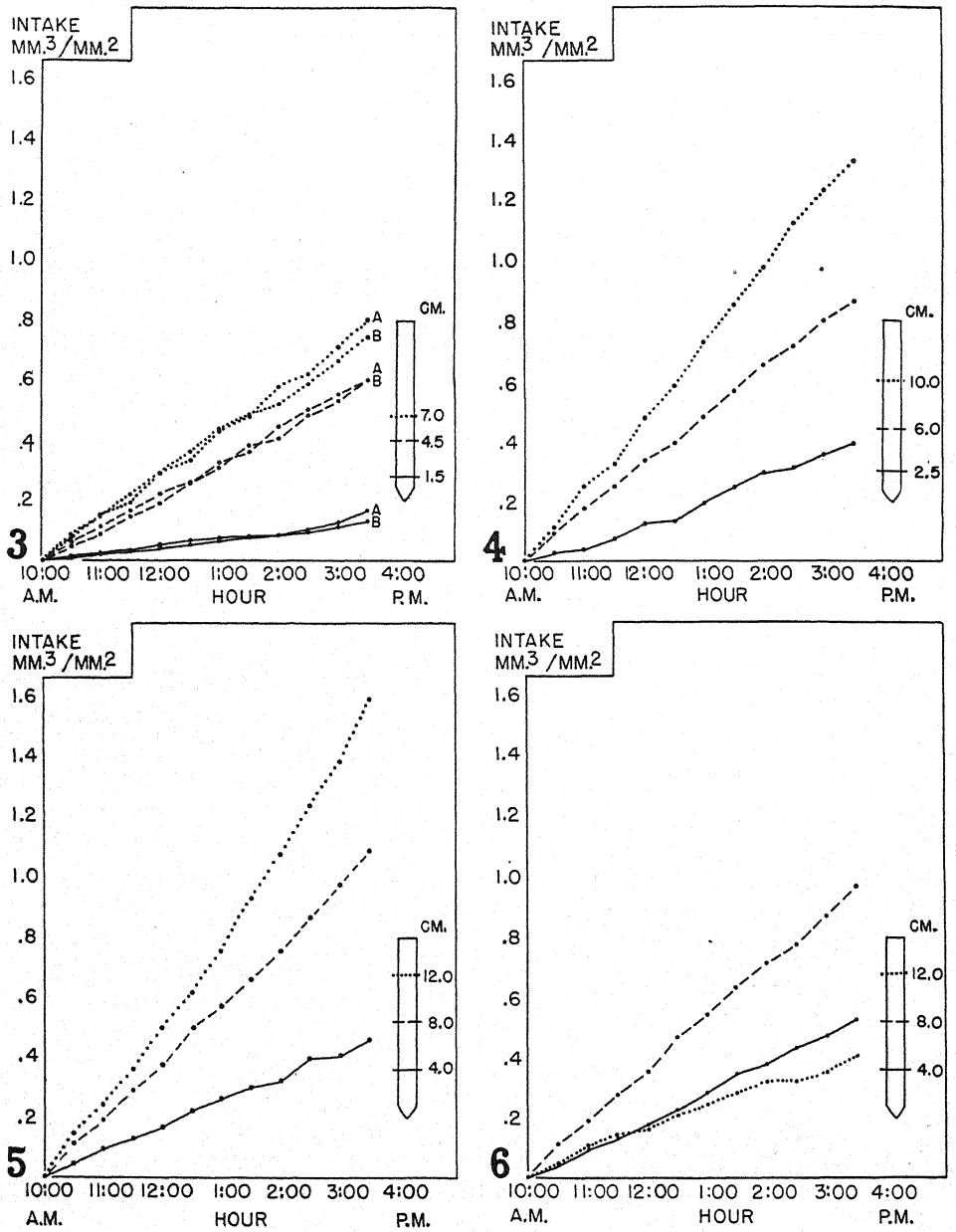
Experiments with corn

The first objective was to measure the rate of entry of water at various levels above the root tip to determine whether an axial gradient existed; and if so, at approximately what level the most rapid rate occurred. In each experiment the rates of entry of water at three levels of the test root were measured simultaneously. By using various triple combinations, a composite picture of the movement of water into the root at successive levels above the tip was obtained.

SERIES I.—Potometers were attached at the 1.5-, 4.5-, and 7.0-cm. levels above the root tip. Readings were made at half-hour intervals from 10 A.M. to 3:30 P.M. Figure 3 shows the results obtained with two representative roots of this series. The intake of water was least rapid at the 1.5-cm. level nearest the root tip, greatest at the 7-cm. level, while the 4.5-zone exhibited an intermediate rate of absorption.

SERIES II.—The results of series I did not necessarily indicate the point of highest rate of absorption, and so the brackets were extended to include a zone 10 cm. from the root tip. Figure 4 illustrates the most common type of gradient obtained. The rate of entry was most rapid at the 10-cm. level farthest from the root tip. In most cases, the rate at the 6-cm. zone was slightly less than at the 7–9-cm. levels. With an occasional root, rather high rates of ingress were observed at the 2–3-cm. region.

In a few instances a second type of gradient was noted in which the rate of entry at the 10-cm. level was slower than at points nearer the root tip, indicating that there may be progressively increasing rates of entry from both the distal and proximal regions of the root which reach a peak at some intermediate point.



FIGS. 3–6.—Rate of entry of water by adventitious roots of corn at various levels above root tip. In each graph the levels measured are designated on the root at the lower right corner.

SERIES III.—When the test levels were set at 4, 8, and 12 cm., two types of gradient were found. In a majority of the roots a gradient was noted like that illustrated in figure 5, in which the proximal zone had the most rapid entry of water, with a decreasing rate of absorption toward the root tip. In other cases the 12-cm. level exhibited a slower rate of intake than the 8-cm. zone, and in a few instances it was less than at the 4-cm. level (fig. 6).

SERIES IV.—In the final series, the top level was established at 14 cm. from the root tip, with the other test zones at 10 and 6 cm. As in series III, the results at the highest level fell into two modal groups. In one group, representing about half the roots tested, there was pronounced reduction in the rate of entry at the 14-cm. level, while in the other there was a slight increase in the rate as compared with the modal group at the 12-cm. level. On the basis of the average for

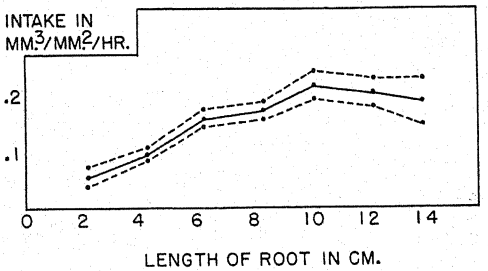


FIG. 7.—Mean rate of absorption of water at various root levels. Standard error indicated by dotted lines.

all roots tested, the rate at the 14-cm. level was less than at the 12-cm. zone, and the highest rate was at 10 cm. from the root tip (fig. 7).

Experiments with sour orange

The most active region for the entry of water by roots is generally regarded as that between the level where the primary vascular elements are elongating and the level of complete suberization of the walls of the epidermis. This is roughly comparable with the zone of the root that produces root hairs.

The foregoing experiments with corn

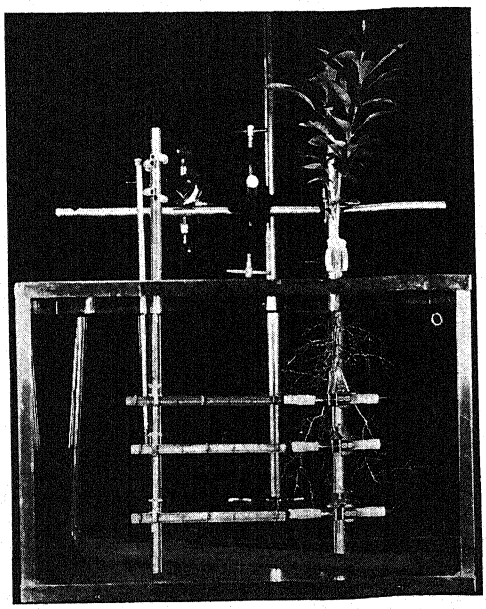


FIG. 8.—Final assembly with 2-year-old sour orange seedling. Potometers attached to two roots; lowest one is the check.

support this point of view, but there is also the possibility of entry of water through hypodermal cells and root lenticels in older portions of the root. Although the lenticel is often regarded as primarily concerned with aeration, there is no structural reason why it should not provide an avenue of entry for water.

To test this possibility, sour orange seedlings 2 years old were used. Potometers were attached to older portions of the roots, in most instances above points where lateral roots had diverged (fig. 8). The structure of a young root lenticel is illustrated in figure 9, and that of an older one in figure 10. Some old suberized root hairs persist where the epider-

mis is still intact, but it is doubtful whether they are functional, owing to the condition of their walls. Structurally the parenchymatous cells of the lenticel would permit the ingress of water, and they appear to be sufficiently numerous

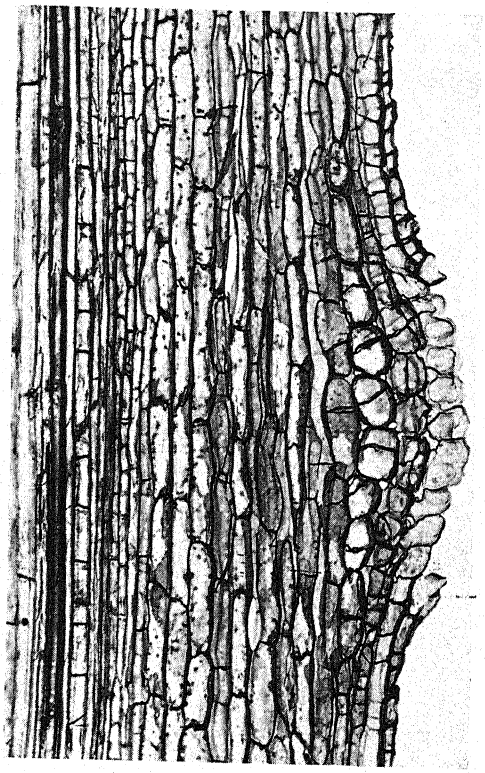


FIG. 9.—Longisection of portion of orange root showing development of lenticel. Periclinal divisions of hypodermal cells which function as phellogen are shown and also anticlinal divisions of underlying parenchymatous cells of cortex.

to account for the rate of absorption observed.

Absorption curves obtained from four roots of sour orange are shown in figure 11. The rate of entry of water was much slower than that for the young active roots of corn, but it approximates that of the 2–3-cm. zone just above the root cap in that plant. When the total area of the old root surfaces is taken into account, it is evident that considerable quantities of water can be absorbed through them laterally, even though the rate is slow. The area, however, would be much less than that of many small roots.

Discussion

The experiments with corn indicate that there is an ascending gradient of rate of entry of water from the tip toward the proximal portion of the root, which

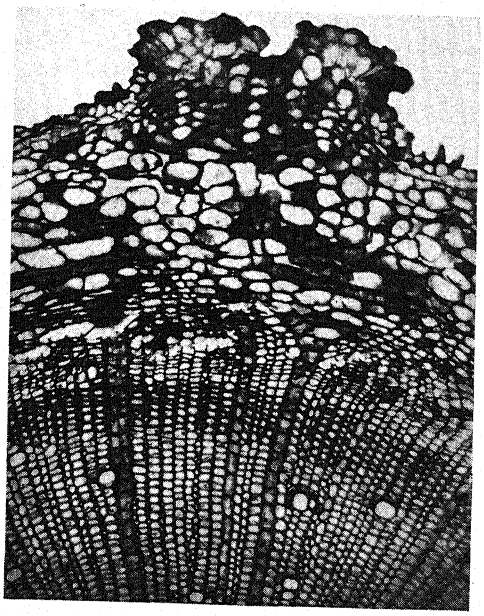


FIG. 10.—Transection of portion of older root of sour orange showing a lenticel. A few suberized root hairs at right.

reaches a maximum about 10 cm. from the root cap. In most cases the rate decreases above that point, and at the 12–14-cm. level it may approximate that of the 2–4-cm. level. The rate in the zone just above the root cap is relatively constant, but there is considerable variation at higher levels when different roots are compared. This may be explained in

part by differences in leaf area, root area, and metabolic activity of the test plant. As ROSENE has pointed out, another consideration is the relative degree of maturity of the root. If two roots of the same length but different age were tested, the rates of water intake would probably be different, owing to the difference in the maturity of zones at equal distances from the root tips. Thus differential growth rate may account for some of the variation in intake at the intermediate and higher levels. This variation would tend to be less pronounced at the lowest level, since—regardless of growth rate—the zone immediately above the root cap would be more or less uniform with respect to its developmental status.

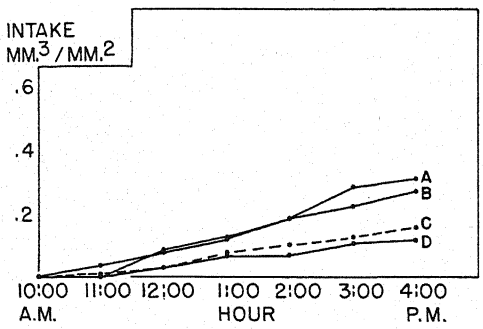


FIG. 11.—Rate of entry of water by four roots of sour orange in which lenticels had formed and epidermis was probably nonfunctional with respect to ingress of water. Roots *A*, *B*, and *D* from same plant.

These studies are in general agreement with those of HÖHN (4), SIERP and BREWIG (10), and ROSENE (7, 8), who have concluded that the most active entry of water occurs in the terminal 10 cm. of the root and that there is less absorption at its apical region than in the more basal zones. With onion, ROSENE also found a two-way gradient comparable with the results reported here for corn.

The ability of older portions of lateral roots to absorb water and nutrient ions has been given little consideration in studies of root-soil relationship. CRIDER (2) has reported absorption of nitrate and phosphate by older portions of *Citrus* and *Vitis* roots after the young unsuberized portion had been excised and the end sealed off. KRAMER (5) has demonstrated the absorption of water by dead root systems, reviewing earlier studies of this nature. NIGHTINGALE (6), working with apple and peach, has stated that "there must obviously have been considerable absorption of water through old large roots."

The preliminary results reported here indicate that older suberized roots of the sour orange do absorb a measurable amount of water, comparable with that taken in by the less active zones of a young root. In view of scattered field observations which indicate that *Citrus* trees have few, if any, young active white roots at certain seasons of the year, the water that enters through the older roots probably constitutes a significant fraction of that utilized. It seems probable that for many perennial plants, especially those with evergreen foliage, intake of water by older portions of young, or even old roots, occupies an important place in their normal soil-water relations.

Summary

1. A potometric device for the quantitative determination of water movement into roots is described. The apparatus is designed so that it may be attached to any part of the root.

2. Experiments with young roots of corn indicate that the rate of water entry increases from the zone proximal to the root cap to a maximum at a point 10 cm. from the root tip. In most cases the rate decreases above this level in roots more than 10 cm. in length.

3. The lateral entry of water into older portions of *Citrus* roots is measurable, and they probably provide the major avenue of ingress during certain seasons of the year. The root lenticels may be the structures through which water most readily enters older portions of the root.

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LITERATURE CITED

1. BREWIG, A., Die Regulationserscheinungen bei der Wasseraufnahme und die Wasserleitgeschwindigkeit in *Vicia faba*-Wurzeln. Jahrb. wiss. Bot. **82**:803–828. 1935.
2. CRIDER, F. J., Selective absorption of ions not confined to young rootlets. Science **78**:169. 1933.
3. GREGORY, F. G., and WOODFORD, H. K., An apparatus for the study of the oxygen, salt, and water uptake of various zones of the root, with some preliminary results with *Vicia faba*. Ann. Bot. (N.S.) **3**:147–154. 1939.
4. HÖHN, K., Die Bedeutung der Wurzelhaare für die Wasseraufnahme der Pflanzen. Zeitschr. Bot. **27**:529–564. 1934.
5. KRAMER, P. J., The intake of water through dead root systems and its relation to the problem of absorption by transpiring plants. Amer. Jour. Bot. **20**:481–492. 1933.
6. NIGHTINGALE, G. T., Effects of temperature on growth, anatomy, and metabolism of apple and peach roots. BOT. GAZ. **96**:581–639. 1935.
7. ROSENE, HILDA F., Distribution of the velocities of absorption of water in the onion root. Plant Physiol. **12**:1–19. 1937.
8. ———, Comparison of rates of water intake in contiguous regions of intact and isolated roots. Plant Physiol. **16**:19–38. 1941.
9. ROSENE, HILDA F., and LUND, E. J., Results obtained by a new technique for the measurement of water absorption in onion roots. Meeting of Proc. Amer. Soc. Plant Physiology, Berkeley, California. June, 1934.
10. SIERP, H., and BREWIG, A., Quantitativ Untersuchungen über die Wasserabsorptionzone der Wurzeln. Jahrb. wiss. Bot. **82**:99–122. 1935.

EFFECTS OF PLATINUM CHLORIDE ON BEAN AND TOMATO[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 547

CHARLES L. HAMNER

(WITH TWO FIGURES)

Introduction

When bean and tomato plants were grown in sand culture to which a nutrient solution containing various amounts of platinum chloride had been added, marked differences in growth occurred. These were associated with differences in anatomical structure, osmotic concentration of sap, rate of wilting, and calcium and phosphorus content of the plants.

The experiments were conducted in the greenhouses of the University of Chicago in the spring of 1942. Red kidney bean and Bonny Best tomato were used. In all the experiments glazed crocks and quartz sand were employed. The platinum chloride, $H_2PtCl_6 \cdot 6H_2O$, was obtained from the Eastman Kodak Company. In general five seeds were planted in each $1\frac{1}{2}$-liter crock filled with the sand. After the seeds had germinated and the primary leaves were expanded, the plants were thinned for uniformity to two per pot (fig. 1*A*) and treated immediately after thinning. The pots were arranged in rows of eight across a greenhouse bench. Each row was regarded as a unit and every pot in it given the same treatment; the lots were randomized and each treatment replicated seven times.

Investigation

EXPERIMENT I.—The seeds were planted May 16, divided into lots and treated on May 26, May 28, June 1, and harvested June 4. The several lots were treated with the following concentrations of platinum chloride: 15^{-5}, 9^{-5}, 3^{-5}, 3^{-6}, 3^{-7}, 3^{-8}, 3^{-9}, and controls to which none was added. The platinum chloride was added to the nutrient solution, which consisted of $Ca(NO_3)_2$ 0.00060 mol., $MgSO_4$ 0.00045 mol., and KH_2PO_4 0.00045 mol. per liter, with traces of micronutrients added. The controls received 250 cc. of nutrient solution at the time of treatment and the treated plants the same amount with the necessary quantity of platinum chloride dissolved in it.

Eight days after the first treatment the plants were harvested. Wet weights of the tops were taken. They were then dried at 80° C., the ash determined, and analyses made for calcium (1) and phosphorus (2, 3).

Concentrations of 15^{-5} applied at an early stage in the growth resulted in failure of the first set of trifoliate leaves to develop, and growth was markedly arrested; at a concentration of 3^{-5} this first set of leaves expanded but soon became chlorotic (fig. 1); a concentration of 3^{-6} or less had no significant effect.

Table 1 shows the inhibitory effect on accumulation of dry weight of the platinum chloride, especially at the higher concentrations. Plants in treatments 15^{-5}, 9^{-5}, and 3^{-5} (table 1) were less succulent. No plants were killed, even at the strongest concentration. All accumulated some dry weight.

The amount of ash in the plants

[1] This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

treated with higher concentrations of platinum chloride was less than in the controls, and the amount of calcium per gram of ash and per plant was less in the treated plants (table 2). The difference in total amount of phosphorus was not so great between treated and untreated plants as in the case of calcium, but the relative amounts varied. Some of the treated lots had a greater percentage of phosphorus when compared with the initial controls. Apparently platinum chloride, by stunting the plant, inhibits the normal intake of calcium; but the phosphorus relationships are influenced relatively less.

EXPERIMENT II.—This set of bean plants was grown and treated as previously described. On these plants studies on transpiration, osmotic concentrations, cellular details, and wilting were based.

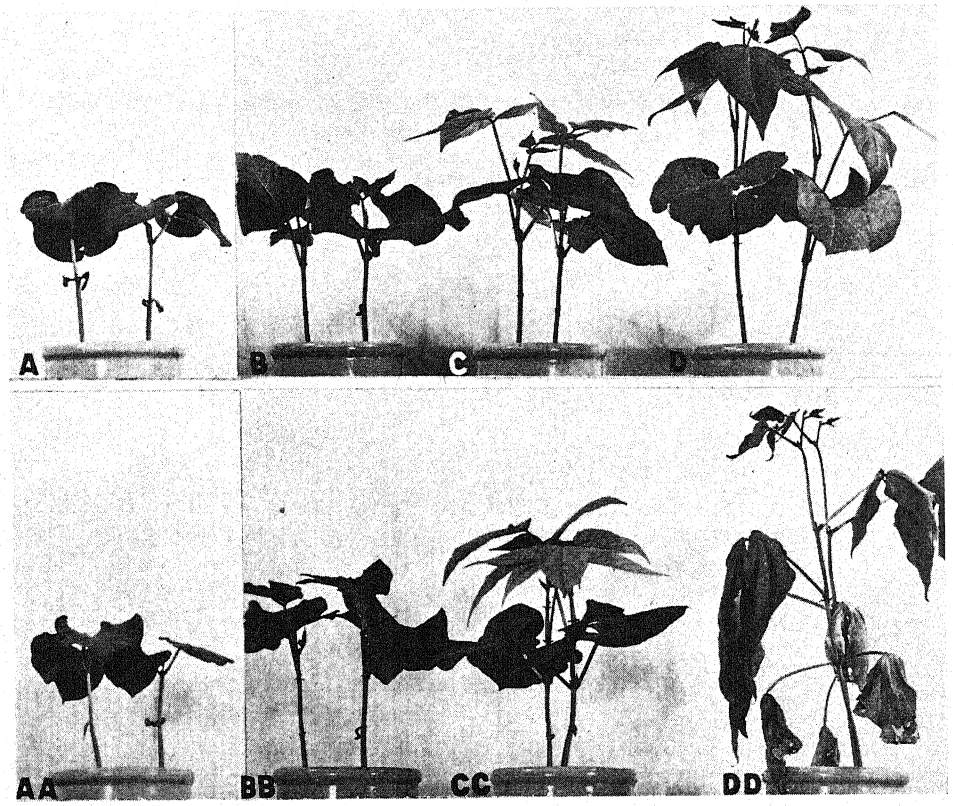


FIG. 1.—*A*, *AA*, bean plants at beginning of experiment; *B*, *C*, *D*, 8 days later. *B*, treated with 15^{-5} and *C* with 3^{-5} solution of platinum chloride; *D*, control. *BB*, *CC*, and *DD*, plants similarly treated 3 days later, watering having been discontinued. Plants receiving the platinum chloride grew more slowly and wilted much less readily.

The amount of transpiration of the treated and untreated plants was determined by weighing. Two plants were taken from each of the following lots: 15^{-5} platinum chloride, 3^{-5} platinum chloride, and controls, immediately after

the third treatment, which was given 6 days after the first treatment. Cork pads were placed over the tops of the pots to permit most of the stem and all of the tops to be exposed to the air. The pads were sealed to the pots with beeswax and the entire apparatus weighed. Six subsequent weighings were made over a period of 3 days.

Table 3 shows the effect of platinum chloride on transpiration. Plants receiving platinum chloride at concentrations of 15^{-5} and 9^{-5} lost less than one-fifth as much water as the controls, but the leaf area and the loss per square centimeter were greater in the controls.

The osmotic concentration of the leaf tissues was determined on plants grown in this experiment by the strip method as described by MEYER and ANDERSON (2). Small 1-mm. squares of leaf tissue were plunged into a known molecular concentration of sugar and examined under the microscope to determine the isotonic solution. The lots of plants which received concentrations of 15^{-5} and 3^{-5} platinum chloride and the controls were examined. Examinations were made 8 days after the first treatment. Squares cut near the center of the primary leaves, near the midrib, were used. The tissues of the primary leaves from control plants averaged below 20 atmospheres while those from the plants treated with 15^{-5} concentration averaged above 40 atmospheres, a difference great enough to be significant, despite possible errors in-

TABLE 1

WET AND DRY WEIGHT (IN GRAMS) AND PERCENTAGE WATER OF BEAN PLANT TOPS TREATED WITH VARYING CONCENTRATIONS OF PLATINUM CHLORIDE IN THE NUTRIENT SOLUTION

CONCENTRATION IN NUTRIENT SOLUTION	WET WEIGHT PER PLANT (GM.)	DRY WEIGHT PER PLANT (GM.)	WATER (%)	INCREASE IN DRY WEIGHT OVER INITIAL CONTROL (GM.)
15^{-5}	3.62	0.506	86.1	0.179
9^{-5}	4.51	0.629	86.1	.302
3^{-5}	6.37	0.800	87.5	.473
3^{-6}	11.75	1.182	90.0	.855
3^{-7}	11.47	1.160	89.9	.833
3^{-8}	11.73	1.207	89.8	.880
3^{-9}	11.87	1.234	89.7	.907
Control	11.44	1.217	89.2	0.890
Initial control*	.....	0.327	.....	.....

* At time of first treatment.

TABLE 2

WEIGHTS AND ANALYSES OF TREATED BEAN PLANTS

PLATINUM CHLORIDE CONCENTRATION IN NUTRIENT SOLUTION	TOTAL ASH PER PLANT (GM.)	CALCIUM PER GRAM ASH (%)	TOTAL CALCIUM PER PLANT (MG.)	PHOSPHORUS PER GRAM ASH (%)	TOTAL PHOSPHORUS PER PLANT (MG.)
15^{-5}	0.0503	19.5	9.76	9.6	4.8
3^{-5}	.0864	18.5	16.0	8.8	7.6
3^{-7}	.1484	23.0	34.10	7.04	10.4
Control	.1569	23.1	35.77	7.05	11.06
Initial control*	0.0413	16.6	6.86	8.2	3.38

* At time of first treatment.

TABLE 3

AMOUNT OF WATER TRANSPIRED FROM BEAN PLANTS STARTING 6 DAYS AFTER INITIAL TREATMENT, EXPRESSED IN GRAMS, OVER PERIOD OF 3 DAYS

CONCENTRATION IN NUTRIENT SOLUTION	WATER LOSS PER PLANT (GM.)	LEAF AREA PER PLANT (SQ. CM.)	WATER LOSS PER 100 SQ. CM. OF LEAF AREA (GM.)
15^{-5}	35	142	24.6
9^{-5}	32	160	20.0
3^{-5}	75	200	37.5
Control	191	300	63.6

herent in the method used for making the determinations.

Fresh and paraffin sections were also made of leaves of comparable lots of plants. The leaves were fixed in Navashin's solution, imbedded in paraffin by the tertiary-butyl-alcohol method, and sectioned. These sections showed a thickening of the walls of the leaf tissue, fewer intercellular spaces, more stomata closed, and greater density of protoplasm in the case of the plants which had received the platinum chloride.

Some of the plants were supplied no water or nutrient solution after the eighth day. These showed a striking response. Those treated with platinum chloride at concentrations of 15^{-5}, 9^{-5}, and 3^{-5} did not wilt so rapidly as the controls. Some of the treated plants remained turgid for 20 days after watering ceased, whereas in all cases controls wilted within 2 days. Most of the treated plants remained turgid for a week after the controls had wilted.

EXPERIMENT III.—This experiment was run to determine the effect of the addition of platinum chloride on plants grown in the dark. For this purpose five seeds of bean were planted in quartz sand in 1½-liter crocks and placed at once in a darkroom. After the plants had emerged above the sand and were approximately 3 inches tall, two were retained in each pot and the other discarded. Plants in six of the crocks received 15^{-5} platinum chloride in the nutrient solution as a single treatment, and six crocks were left untreated, receiving nutrient solution only. Both treated and untreated plants remained continuously in the dark for several days. During this period the hypocotyls and epicotyls of the treated plants failed to elongate, yet the plants were not killed. The effect was mainly that of stopping elongation.

EXPERIMENT IV.—Bean plants were planted in sand and thinned as previously described. They were given nutrient solution until they were mature enough to flower. Six pots were then treated with 15^{-5} platinum chloride in the nutrient solution and six left as controls. In all, there were three applications to each of the pots over a period of 7 days. No further addition of the solution or water was given the treated and control plants, and the rate of wilting was observed. The treated plants remained turgid several days longer than the controls, as was true in other experiments.

EXPERIMENT V.—In this experiment five bean seeds were planted in 5-inch porous pots filled with soil instead of sand. After germination the plants were thinned to two per pot. Six pots received 15^{-5} platinum chloride in solution after the primary leaves had fully expanded, and six were left as controls. Three applications on alternate days were given. The plants were examined for gross appearance, then all liquid was withheld and the plants allowed to wilt. Bean plants grown in soil apparently do not respond in the same manner to low concentrations of platinum chloride when it is supplied in nutrient solution added to the soil. No visible differences in appearance or amount of growth were observed, and no differences in wilting response were noted.

TOMATO

Tomato seedlings were grown in sand in flats. They were transplanted when about 2 inches tall to 1½-liter glazed crocks filled with quartz sand, where they were allowed to grow for 2 weeks before being treated. The two plants in each pot were about 4 inches tall when treated with platinum chloride (fig. 2*A*). The plants were treated in the same

manner and with the same concentrations of platinum chloride as in the experiment with bean (experiment I).

Plants treated with concentrations of platinum chloride of 15^{-5}, 9^{-5}, and 3^{-5} showed a response similar to bean. Growth was inhibited (table 4) and the percentage water decreased slightly in the treated plants. No response was noted for treatments at the lowest concentration. The plants treated with a concentration of 15^{-5} showed some chlorosis in the lower leaves (fig. 2). The plants treated with 15^{-5}, 9^{-5}, and 3^{-5} platinum chloride remained turgid much longer than the controls when water was withheld. The controls wilted 1 day after water was withheld; the treated plants remained turgid for more than a week.

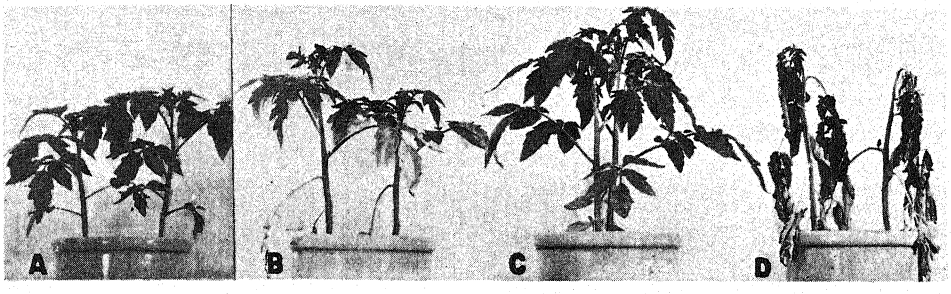


FIG. 2.—Tomato plants: *A*, at time treatments were begun, the others 10 days later, having received no water for the last 2 days. *B*, treated with 15^{-5} and *C*, with 3^{-5} platinum chloride added to nutrient solution; *D*, control. Effects similar to those received with bean.

TABLE 4

WET AND DRY WEIGHT (IN GRAMS) AND PERCENTAGE WATER OF TOMATO PLANTS TREATED WITH VARYING CONCENTRATIONS OF PLATINUM CHLORIDE IN THE NUTRIENT SOLUTION

CONCENTRATION IN NUTRIENT SOLUTION	WET WEIGHT PER PLANT (GM.)	DRY WEIGHT PER PLANT (GM.)	WATER (%)
15^{-5}	4.72	0.612	87.03
9^{-5}	4.66	0.600	87.13
3^{-5}	6.98	0.875	87.45
3^{-6}	10.95	1.31	88.05
3^{-7}	10.84	1.25	88.47
3^{-8}	10.96	1.32	88.65
3^{-9}	10.99	1.38	87.90
Control	10.98	1.33	87.90

RESULTS WITH CUTTINGS

In a few additional experiments tomato and bean seedlings were cut off near the surface of the soil and placed in solutions of platinum chloride of the following concentrations: 15^{-5}, 3^{-5}, and controls. The stems were cut under water and placed in small vials containing 30 cc. of the respective solutions. Five seedlings were used for each treatment. The plants promptly showed symptoms of toxicity. The tomato is particularly susceptible to platinum injury. Black spots similar to the symptoms of ammonia toxicity appear in the leaves shortly after the cuttings are placed in the solution.

Summary

1. Bean plants grown in sand at concentrations of 15^{-5}, 9^{-5}, 3^{-5} platinum chloride, $H_2PtCl_6 \cdot 6H_2O$, are inhibited in growth, have smaller leaf area, higher osmotic pressure, have a lower transpiration rate, resist wilting much longer than controls, and are less succulent.

2. Bean plants grown in sand in the dark at platinum chloride concentrations

of 15^{-5}, 9^{-5}, 3^{-5} are inhibited in stem and hypocotyl elongation.

3. Bean plants grown in soil to which platinum chloride at a concentration of 15^{-5} was added fail to show any response.

4. Tomato plants grown in sand supplied with platinum chloride at concentrations of 15^{-5}, 9^{-5}, and 3^{-5} are inhibited in growth, have chlorotic lower leaves, and resist wilting longer than controls.

5. When the cut ends of severed bean or tomato plants are placed in dilute concentrations of platinum chloride, they soon show symptoms of toxicity and are killed.

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LITERATURE CITED

1. METHODS OF ANALYSIS, A.O.A.C. 4th ed. 1935.
2. MEYER B. S., and ANDERSON, D. B., Plant physiology. Van Nostrand Co. 1939.
3. WALL, M. E., Microdetermination of some constituents of plant ash. Plant Physiol. 15:537–545. 1940.

DEVELOPMENT OF THE NODE IN RICINUS COMMUNIS

MARY E. REYNOLDS

(WITH NINE FIGURES)

Introduction

The structure of the node in certain monocotyledons such as grasses and palms is extremely complex. The intricate vascular septum which is generally present differs in origin and appearance in different plants. In the mature node of *Zea mays*, for example, this reticulum originates by anastomosis of branches both of the entering leaf traces and of the vascular bundles descending from the younger leaves (1). In *Spartina townsendii*, in contrast to this, LAUDER-THOMSON (2) concludes that only the "so-called cauline" bundles are responsible for the network formation.

In the hollow-stemmed dicotyledon, *Ricinus communis*, the anatomy of the node is equally complex. A similar vascular meshwork imbedded in parenchyma tissue is present, but it differs in position from the preceding monocotyledon types. The nodal septum of *Ricinus* lies somewhat obliquely in the stem above the level of the entering leaf traces, which are therefore not directly connected with the network. The general anatomy of *Ricinus* has been outlined recently by SCOTT (5, 6), but the structure of the node is not discussed in detail. In the present paper the development of the vascular septum is followed through successive internodes.

Material and methods

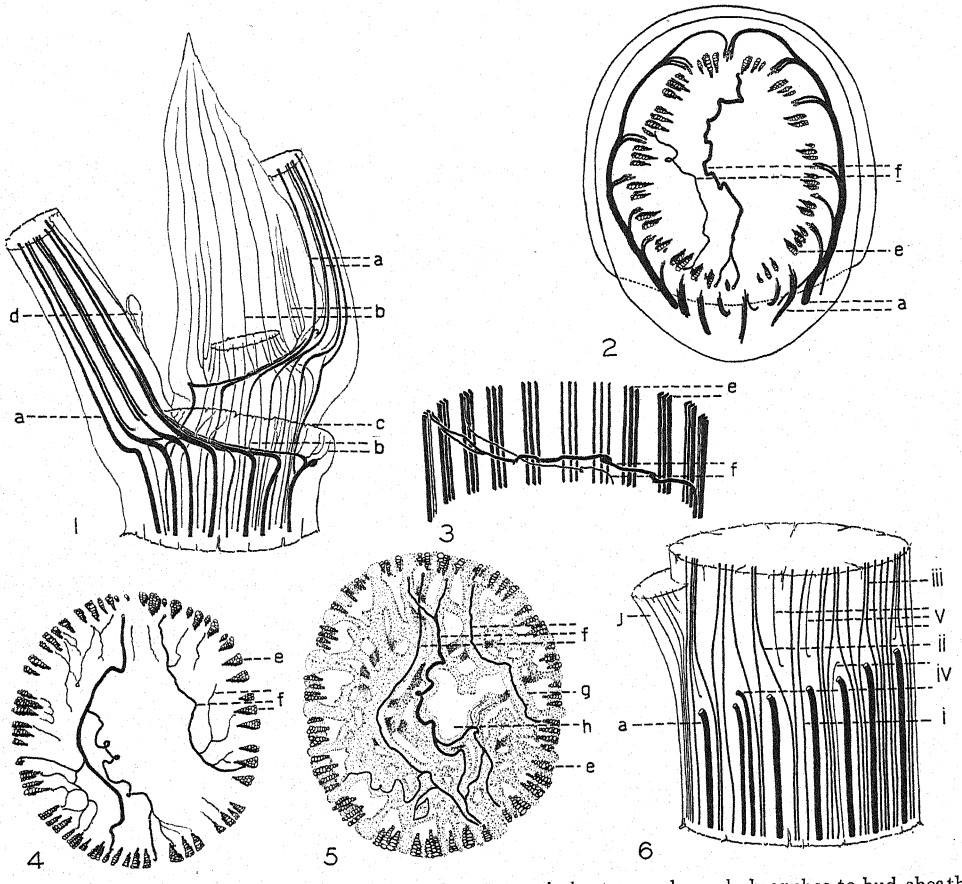
The habit of *Ricinus communis* (4) requires no detailed description. It grows abundantly in southern California as a hardy perennial weed or garden plant, so that seedlings and plants in various stages of growth are readily obtainable throughout the year.

Reconstruction of the path of the numerous individual leaf and stipular traces from only transverse and longitudinal serial sections is difficult and tedious. In order to obtain a three-dimensional picture of the vascular pattern as a whole, the following clearing and staining method proved most useful. Seedling axes and actively growing shoots, from the tip to the fourteenth node inclusive, were cleared in chloral hydrate. At room temperature complete clearing may take 2 months, but oven-heating at 50° C. may reduce this time to a week or two, depending upon the age of the tissue. When the material is sufficiently clear it is rinsed in water and stained in phloroglucin and HCl, then mounted in pure glycerin. Staining of the lignified tissues requires 15–30 minutes, depending upon the thickness of the material. Sections stained in this manner have retained their color for over a year.

Observations

In studying the development of the node it is necessary to consider the path of the vascular bundles in the stem and in the leaf base. In the sunflower, with a phyllotaxy of 2/5, or less frequently 3/8, one relatively simple pattern of dicotyledon structure has been clearly traced (3, 7). Three leaf traces enter the stem at each node and continue vertically downward until they reach the level of the next leaf below on the same orthostichy;

for instance, the sixth leaf in a shoot of 2/5 phyllotaxy. Here they may either fork or swing to one side or the other of the incoming leaf traces and sometime sultant pattern is therefore much more complex. The differentiating axial vascular bundles are necessarily deflected sooner from the perpendicular. They



FIGS. 1–6.*—Fig. 1, bud sheath and axis: leaf traces encircle stem and supply branches to bud sheath. Fig. 2, tenth node, septum: entering and encircling leaf traces surround axile vascular bundles seen in transection. Two vascular strands, lignified (spiral) vessels traverse septum; provascular tissue not shown. Fig. 3, tenth node, profile: vascular strands continue downward after crossing septum. Fig. 4, eleventh node, septum: further lignification gives rise to vascular network. Fig. 5, twelfth node: secondary thickening (stippling) completes nodal mesh; primary spiral vessels (solid black) still visible. Fig. 6, twelfth node, profile (semidiagrammatic): path of vascular bundles summarized. They may either: i, run vertically downward; ii, be deflected right or left; iii, diverge around entering traces; iv, pass inward to traverse septum; v, emerge and descend after crossing septum.

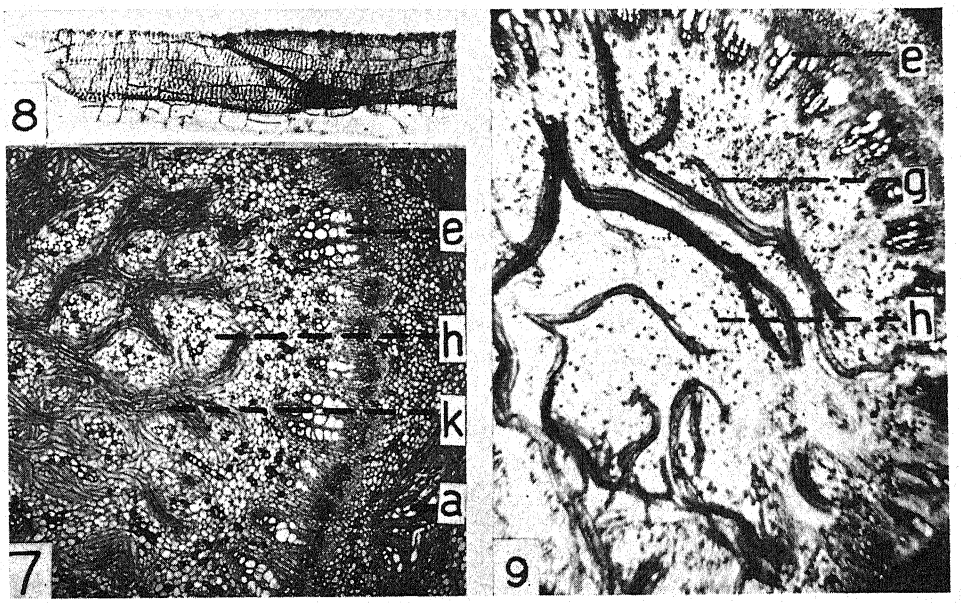
* *a*, leaf trace; *b*, sheath trace; *c*, sheath scar; *d*, extrafloral nectary; *e*, axial vascular bundle; *f*, transverse vascular strand; *g*, secondary vascular strand; *h*, pith; *j*, bud; *k*, provascular network.

thereafter fuse below with other vascular bundles.

In *Ricinus* the phyllotaxy is also 2/5, or less commonly 3/8; but instead of three traces, as in the sunflower, they number ten to twenty-three, and the re- may either merge sooner with other axial bundles or be deflected horizontally across the node to form the vascular septum.

The first eight nodes of the growing shoot are inclosed within the conical

caducous stipular bud sheath of the ninth leaf, which is not encircled by the sheath. The vascular supply of the bud sheath consists of ten to thirty-five veins, depending upon the size of the sheath (fig. 1). The veins run parallel, branching only near the base and tapering gradually toward the sheath tip. They are small, consist of one or two spiral vessels only, and arise as branches from the smaller entering leaf traces of the ninth leaf.



FIGS. 7–9.—Fig. 7, ninth node, transection; provascular tissue. Fig. 8, secondary pitted vessel segments from nodal septum isolated by maceration: pitted vessel segment has width of 68 μ, length of 272 μ. Fig. 9, twelfth node, transection: fresh material stained with phloroglucin and HCl; spiral and pitted vessels present in nodal network. Lettering as in figs. 1–6.

Primary differentiation of the axis is seen within the bud sheath. Spiral vessels, the first evidence of xylem differentiation, appear first near the base of the third leaf primordium. At this level the provascular network of the septum is already well defined and becomes increasingly prominent in the older nodes. Beyond the bud sheath differentiation of primary xylem elements continues in the axis and is followed later by secondary thickening. Meantime, at the ninth node the first traces of lignification in the septum are evident and intermittent horizontal spiral elements may be observed, while at the tenth node one or more continuous vascular strands traverse the septum (figs. 2, 7). These strands vary in thickness from one to three spiral vessels and are in direct continuity with the bundles of the axial vascular cylinder (fig. 3). The elements which compose the strands are similar to those of the primary stem. The coenocytic development of the vessels is evident (5) and sieve tubes of the usual type are present in the accompanying phloem. Further lignification occurs in the eleventh septum (fig. 4). Here the network is practically complete and occasional reticulate elements are present in addition to the spiral vessels.

Secondary thickening is initiated in both the axis and the septum at the level of the twelfth or thirteenth node and re-

sults in the heavy woody reticulum seen in the older stem (figs. 5, 9). In this network the initial spiral vessels are still clearly visible, together with reticulate elements, pitted vessel segments, xylem fibers, and lignified parenchyma (fig. 8). The increased thickness of the vascular tissue results in some compression of the adjacent parenchyma. The cells of the ground tissue as a whole remain alive, however, and function in the storage of starch, fat, and calcium oxalate.

In a stem as complex as *Ricinus* it proved practically impossible to follow each individual bundle to its ultimate conclusion. Of the numerous traces entering the stem at any one node, the three median turn directly downward while the laterals more or less encircle the stem as girdling traces before assuming a vertical direction (figs. 1, 2). All traces appear to continue through two or three internodes before they are blocked by incoming vascular bundles from the lower leaves. At this level the traces diverge, some passing horizontally inward to the septum while the others are deflected right or left or divide around the incoming strands (fig. 6). This diversion from the vertical may result in fusion with adjacent bundles, but it is usually possible to trace any one major strand throughout two or three more internodes. Thus leaf traces may be followed altogether through five internodes before becoming obscured by secondary thickening. The fate of the first-formed horizontal traces, already mentioned, is clearly indicated (figs. 2, 3). After crossing the septum, they swing downward and sooner or later fuse with other axial bundles. The actual origin of these traces was not determined, but it would appear possible that all leaf traces take part in the formation of at least one nodal septum.

Summary

1. The differentiation of the complex vascular network of the node in *Ricinus communis* is traced throughout the stem apex.

2. The development of the vascular elements of the septum in general parallels that in the axis. The network differentiates from provascular strands. Spiral vessels in the axis precede those in the septum, while secondary thickening is initiated in both at the same time.

3. It appears that leaf traces entering the stem run downward through only two internodes without change. Thereafter they may either anastomose, diverge right or left of the incoming traces and continue downward, or be deflected horizontally across the nodal septum. Thus it is possible that all leaf traces take part in the formation of at least one nodal network.

This investigation was carried on under the direction of Professor FLORA M. SCOTT, to whom the writer is indebted for helpful suggestions and criticisms.

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LITERATURE CITED

1. EVANS, A. T., Vascularization of the node in *Zea mays*. BOT. GAZ. **85**:97–103. 1928.
2. LAUDER-THOMSON, I., The nodal anatomy of *Spartina townsendii*. Trans. Proc. Bot. Soc. Edinburgh **31**:262–268. 1932–1933.
3. PRIESTLEY, J. H., and SCOTT, L. I., Vascular anatomy of *Helianthus annuus*. Proc. Leeds Phil. Soc. (Sci. Sect.) **3**:159–173. 1936.
4. ROSANOFF, S., Über die Krystalldrüsen im Marke von *Kerria japonica* DC. und *Ricinus communis*. Bot. Zgt. **23**:329–330. 1865.
5. SCOTT, FLORA M., Differentiation of spiral vessels in *Ricinus communis*. BOT. GAZ. **99**:69–79. 1937.
6. ———, Differentiation of calcium oxalate crystals in *Ricinus* in relation to tissue differentiation and the presence of other ergastic substances. BOT. GAZ. **103**:225–246. 1941.
7. THODAY, D., On the organization of growth and differentiation in the stem of the sunflower. Ann. Bot. **36**:489–510. 1922.

VARIATIONS IN CHROMOSOMAL ASSOCIATION AND BEHAVIOR DURING MEIOSIS AMONG PLANTS FROM OPEN-POLLINATED POPULATIONS OF DACTYLIS GLOMERATA[1]

W. M. MYERS AND HELEN D. HILL[2]

Introduction

MÜNTZING'S (6, 7) report that *Dactylis glomerata* L. (orchard grass) behaved cytologically as an autotetraploid has been verified by both the cytological results obtained by MYERS and HILL (13, 14) and the genetical data obtained by MYERS (9, 10, and unpublished). Among three plants of this species, MYERS and HILL (13, 14) found considerable differences in frequency of quadrivalents at diakinesis and in percentages of metaphase I sporocytes with univalents, of anaphase I sporocytes with lagging and dividing univalents, and of quartets with micronuclei. Since no valid estimate of error was available, the statistical significance of these differences could not be determined. A knowledge of the range of variation in chromosomal behavior during meiosis among normal plants from open-pollinated populations of an autotetraploid species is of considerable importance. Furthermore, the discovery of plants differing significantly in this regard would provide valuable material for an analysis of the interrelations of irregularities manifest during different stages of meiosis and for an evaluation of the relative importance of such irregularities in conditioning the germinal instability of the species. A preliminary report of the results presented in this paper has been published in abstract (15).

Material and methods

Twenty plants which had been used as parents in an inbreeding program, and which were known to be euploid ($2n = 28$), were selected for this study. Six of the plants were from seed collected in the northeastern United States and the remainder were from various Foreign Plant Introductions.

Clonal increases from these plants were grown in 4-inch pots in the greenhouse during the winter of 1939–40. Panicles from all plants were collected and fixed on February 22 and again on February 24. The cytological technique has been described previously (14). Complete data on frequency of quadrivalents, of univalents at metaphase I, of lagging and dividing chromosomes, and of dicentric bridges and acentric fragments at anaphase I, and of micronuclei in the quartets were obtained from twelve plants for both collections. Data on micronuclei were obtained from two additional plants in both collections. Complete data were obtained for each of the remaining plants from only one collection.

Experimental results

DIAKINESIS

Quadrivalent frequency was determined at diakinesis for all plants (table 1). For this purpose, sporocytes were arbitrarily selected in which all chromo-

[1] Contribution no. 36 of the U.S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture, in cooperation with the northeastern states.

[2] Geneticist and Agent, respectively.

somes were associated as bivalents, quadrivalents, or both. No sporocyte was observed with an association of more than four chromosomes. Among the sporocytes from each plant there was a wide range in number of quadrivalents. Cells with fourteen bivalents were found in four plants while cells with seven quadrivalents were observed in eleven plants. In each plant the frequency distribution of sporocytes with various numbers of quadrivalents between these two extremes was unimodal.

Among the twelve plants for which lata from both collections were available the average number of quadrivalents per sporocyte ranged from 3.42 to 4.39. An analysis of variance (table 2) indicated that the differences among plants were statistically significant; F was greater than F for P of 0.05 (18, table 35). In addition, variation between days of collection was significant (P = less than 0.05). Among the plants with data from a single collection, OG 6(12) had an average of 2.42 quadrivalents. On the basis of the error variance shown in table 2, it is probable that this plant is significantly lower than any among the twelve with data from duplicate determinations. The

TABLE 1

NUMBER OF SPOROCYTES AT DIAKINESIS WITH VARIOUS COMBINATIONS OF BIVALENTS AND QUADRIVALENTS IN TWENTY PLANTS OF DACTYLIS GLOMERATA

PLANT NO.	NO. OF SPOROCYTES SHOWING INDICATED ASSOCIATION								TOTAL NO. OF CELLS	AVERAGE NO. OF QUADRIVALENTS
	^{14}II	$^{1}IV+^{12}II$	$^{2}IV+^{10}II$	$^{3}IV+^{8}II$	$^{4}IV+^{6}II$	$^{5}IV+^{4}II$	$^{6}IV+^{2}II$	^{7}IV		
	TWO COLLECTIONS									
OG 7(20)	0	6	14	22	16	17	3	0	78	3.42
OG 48(28)	2	3	16	16	24	11	4	1	77	3.44
OG 48(43)	0	3	21	23	27	17	6	2	99	3.61
OG 9(10)	1	4	14	26	43	15	8	0	111	3.68
OG 48(47)	0	5	18	26	38	21	12	1	121	3.76
OG 2(3)	0	2	10	16	16	13	6	1	64	3.78
OG 48(48)	0	2	6	15	22	14	4	0	63	3.82
OG 2(21)	0	3	9	22	29	19	6	1	89	3.83
OG 42(5)	0	1	5	13	25	14	4	0	62	3.94
OG 2(11)	1	4	7	18	36	35	11	2	114	4.13
OG 2(20)	0	0	3	13	18	18	5	1	58	4.21
OG 48(92)	0	1	5	19	34	36	19	1	115	4.39
	SINGLE COLLECTIONS									
OG 6(12)	1	14	24	22	11	1	0	0	75	2.42
OG 14(13)	2	1	10	16	17	4	3	0	53	3.30
OG 10(12)	0	0	2	3	4	1	0	0	10	3.40
OG 48(49)	0	2	4	11	16	14	6	0	53	3.87
OG 14(11)	0	1	0	8	7	6	2	0	24	3.96
OG 48(172)	0	1	5	17	20	16	2	3	64	3.98
OG 20(16)	0	0	4	14	14	7	5	1	44	4.00
OG 13(22)	0	0	2	7	10	6	4	1	30	4.20
Total	7	53	178	327	427	285	110	15	1420	.....

remaining seven plants with data from a single collection exhibited a range similar to that shown by the twelve with duplicate collections.

METAPHASE I

The plants are arranged in table 3 in ascending order on the basis of percentage of metaphase I sporocytes with univalents. The twelve plants with data from both days ranged from 3.4 to 13.8 per cent of the sporocytes with one or more univalents. In the analysis of variance of these data, the angular transformation, $P = \sin^2 \phi$, recommended by BLISS (1, 2) was used. The values of ϕ were obtained from FISHER and YATES (5, table 12). The differences among plants and between days were significant (P was less than 0.05 and 0.01, respectively). Among the eight plants with data from a single collection, two were lower and one higher than the extremes among the twelve plants. The differences, however, are within the range of random error and are therefore not significant.

In the plants with a low percentage of sporocytes with univalents, two univalents per sporocyte was the maximum observed. On the other hand, in OG 2(21), for example, 1.7 per cent of the sporocytes had four univalents and 0.8 per cent had five univalents. As a result the range among the plants in number of univalents per 100 sporocytes (table 4) was greater than the range in percentage of sporocytes with univalents.

ANAPHASE I

Lagging univalents which were dividing longitudinally were observed in some anaphase I sporocytes in all plants, the percentages of sporocytes with laggards ranging from 1.7 to 34.0 per cent among the twenty plants (table 3). For the twelve plants with duplicate data, the range was from 6.4 to 34.0 per cent. In the analysis of variance, using the angular transformation of the percentages, the difference between days was significant (P = less than 0.05). For the differences among plants F was slightly less than the value of F for P of 0.05, and consequently these differences were of doubtful statistical significance when compared with the interaction of plants × days.

In each of eighteen of the plants, one or more anaphase I sporocytes examined had a dicentric chromatid bridge accompanied by an acentric fragment, the fre-

TABLE 2

ANALYSIS OF VARIANCE OF QUADRIVALENTS PER SPOROCYTE, METAPHASE I SPOROCYTES WITH UNIVALENTS, ANAPHASE I SPOROCYTES WITH LAGGING AND DIVIDING UNIVALENTS, AND QUARTETS WITH MICRONUCLEI. FOR STATISTICAL ANALYSIS, PERCENTAGES WERE TRANSFORMED TO ϕ

Variation due to	No. of quadrivalents			Percentage of MI with univalents			Percentage of AI with laggards			Percentage of quartets with micronuclei		
	D/F	Mean square	F	D/F	Mean square	F	D/F	Mean square	F	D/F	Mean square	F
Plants	11	0.1971	4.05*	11	29.98	3.87*	11	97.31	2.32	13	58.43	2.58*
Days	1	0.3015	6.20*	1	111.37	14.37†	1	332.27	7.93*	1	289.93	12.79†
Plants × days	11	0.0486	.....	11	7.75	.....	11	41.91	.....	13	22.67	.....

* Value of F exceeds that for P of 0.05.
† Value of F exceeds that for P of 0.01.

quency of such sporocytes ranging from 0.5 to 13.8 per cent for the different plants (table 3). In the two plants, OG 6(12) and OG 10(12), in which no bridges and fragments were observed, only forty-two and fifteen anaphase I sporocytes, respectively, were examined. Illustrations of sporocytes with dicentric chromatic bridges and acentric fragments have been presented earlier (14, fig. 2). Most investigators, including the writers, have interpreted such bridges and fragments as resulting from crossing-over within a heterozygous inversion, although RICHARDSON (17) has shown that similar results may be obtained under certain conditions from crossing-over within duplications and translocations.

TABLE 3

FREQUENCY OF QUADRIVALENTS AND OF SPOROCYTES SHOWING INDICATED MEIOTIC IRREGULARITIES IN TWENTY PLANTS OF DACTYLIS GLOMERATA

PLANT NO.	DIAKINESIS		METAPHASE I		ANAPHASE I			QUARTETS	
	NO. CELLS	AVERAGE NO. QUADRIVALENTS	NO. CELLS	PERCENTAGE WITH UNIVALENTS	NO. CELLS	PERCENTAGE WITH LAGGARDS	PERCENTAGE WITH BRIDGES AND FRAGMENTS	NO. CELLS	PERCENTAGE WITH MICRONUCLEI
	TWO COLLECTIONS								
48(47)	121	3.8	311	3.4	89	15.1	2.9	235	4.4
48(92)	115	4.4	341	3.4	227	10.3	5.7	186	6.3
9(10)	111	3.7	344	4.1	351	8.0	3.6	343	8.6
48(28)	77	3.4	204	4.7	255	6.6	5.0	327	5.8
48(48)	63	3.8	399	4.7	184	6.4	6.8	236	9.8
2(11)	114	4.1	324	4.8	110	6.6	5.0	345	6.4
48(43)	99	3.6	341	5.0	297	7.3	6.4	334	8.2
42(5)	62	3.9	307	5.8	186	21.6	10.6	285	19.2
2(3)	64	3.8	332	7.4	218	7.0	4.0	369	8.4
2(20)	58	4.2	323	10.2	243	13.1	7.0	411	10.4
2(21)	89	3.8	315	11.6	173	27.3	2.6	180	14.6
7(20)	78	3.4	400	13.8	195	34.0	0.5	214	25.6
	SINGLE COLLECTIONS								
13(22)	30	4.2	137	0.0	58	1.7	13.8	131	3.0
48(172)	64	4.0	247	3.2	57	8.8	3.5	241	3.3
20(16)	44	4.0	95	4.2	138	11.6	1.4	106	5.7
14(13)	53	3.3	109	4.6	109	4.6	2.2	131	1.5
10(12)	10	3.4	85	5.9	15	20.0	0.0	184	11.4
6(12)	73	2.4	87	6.9	42	16.7	0.0	100	17.0
48(49)	53	3.9	201	7.5	100	24.0	6.0	217	17.0
14(11)	24	4.0	143	16.8	72	30.6	5.6	198	14.6

It may be concluded that at least eighteen out of twenty plants are heterozygous for a chromosomal aberration, probably an inversion. MÜNTZING (8), MYERS and HILL (13, 14), MYERS (11, 12), and ÖSTERGREN (16) have stressed the high frequency of chromosomal aberrations, particularly inversions, in plants of nor-

mally cross-pollinated species. The results reported here add further evidence for that conclusion.

TABLE 4

NUMBER OF UNIVALENTS AT METAPHASE I AND OF LAGGING UNIVALENTS AT ANAPHASE I PER 100 SPOROCYTES; NUMBER OF MICRONUCLEI PER 100 QUARTETS; AND NUMBER OF MICRONUCLEI EXPECTED ON BASIS OF ANAPHASE I LAGGARDS

Plant no.	Total univalents per 100 sporocytes at		$\frac{AI}{MI} \times 100$	No. of micronuclei per 100 quartets		$\frac{O}{E} \times 100$
	MI	AI		Obtained	Expected	
	Two collections					
OG 48(47)..	4.1	23.3	568.3	7.2	46.6	15.5
OG 48(92)..	4.9	11.4	231.6	12.4	22.9	54.2
OG 9(10)...	6.4	11.0	171.6	14.9	22.0	67.9
OG 48(28)..	6.1	8.2	133.9	11.1	16.4	67.7
OG 48(48)..	5.5	7.0	127.9	21.0	14.0	149.6
OG 2(11)...	6.4	8.4	130.8	10.1	16.8	60.1
OG 48(43)..	6.2	9.6	153.3	14.7	19.1	76.9
OG 42(5)...	6.6	31.0	466.8	39.1	62.1	63.0
OG 2(3)....	10.2	8.1	79.1	16.1	16.2	99.6
OG 2(20)...	11.8	14.8	125.8	15.6	29.6	52.5
OG 2(21)...	19.6	45.8	233.2	33.1	91.6	36.2
OG 7(20)...	20.2	71.2	352.9	50.5	142.4	35.4
	Single collections					
OG 13(22)..	0.0	1.7	.....	3.8	3.4	110.8
OG 48(172).	3.6	8.8	241.6	5.0	17.5	28.3
OG 20(16)..	5.3	15.9	303.0	8.5	31.9	26.6
OG 14(13)..	5.5	5.5	100.0	3.3	11.0	29.6
OG 10(12)..	9.4	20.0	212.5	20.1	40.0	50.2
OG 6(12)...	8.0	21.4	266.2	38.0	42.9	88.7
OG 48(49)..	8.0	35.0	439.7	43.3	70.0	61.8
OG 14(11)..	27.3	51.4	188.3	35.8	102.8	34.9

QUARTETS

The amount of chromosome loss was estimated from the frequency of chromatin material remaining in the cytoplasm of the quartets outside the normal nuclei. This chromatin material occurred in some quartets as dark-staining clumps while in others it was organized as a small nucleus indistinguishable in appearance, except for size, from the normal nucleus of the young microspore. For convenience, all these chromatin bodies are called micronuclei. Judging from size and shape, it seems probable that most, if not all, of the micronuclei consisted of single daughter univalents of the equational division of first-division chromosomes.

Among the twenty plants, the frequency of quartets with micronuclei ranged from 1.5 to 25.6 per cent (table 3). An analysis of variance of the data from the fourteen plants with duplicate collections indicated that the differences among plants and between days were significant (table 2). Among the quartets with micronuclei, those with one or two were most common, while those with three or four occurred rather frequently. In the plants with high percentages of quartets with micronuclei, however, more complicated types with five, six, seven, and (rarely) eight micronuclei were observed. Consequently the number of daughter univalents lost per 100 sporocytes was higher (table 4) than indicated by the data on percentages of quartets with micronuclei. The twenty plants ranged from 3.3 to 50.5 daughter univalents lost per 100 sporocytes.

RELATIONSHIPS OF CHROMOSOMAL BEHAVIOR MANIFEST IN DIFFERENT STAGES OF MEIOSIS

Using the average data from the twelve plants with duplicate determinations, correlation coefficients were calculated for all combinations of the four characters: frequency of quadrivalents,

percentage of metaphase I with univalents, percentage of anaphase I with laggards, and percentage of quartets with micronuclei (table 5). Quadrivalent frequency was not significantly correlated with any of the other three characters. The correlation coefficient of metaphase I univalents with anaphase I univalents was statistically significant; P = less than 0.01 (4, table VA). This relationship is not surprising since it has been reported commonly in the literature that metaphase I univalents tend to lag at anaphase I. From table 4 it can be seen that in eight-

TABLE 5

COEFFICIENTS OF CORRELATION BETWEEN MEIOTIC IRREGULARITIES IN TWELVE PLANTS OF DACTYLIS GLOMERATA FOR WHICH DATA FROM TWO COLLECTIONS WERE AVAILABLE

COMPARISON	D/F	r
Quadrivalents with MI univalents	10	−0.06
Quadrivalents with AI univalents	10	−0.12
Quadrivalents with micronuclei	10	−0.21
MI univalents with AI univalents	10	0.72*
MI univalents with micronuclei	10	0.76*
AI univalents with micronuclei	10	0.82*

* Significant according to FISHER'S (4) table VA.

een of the plants there were more anaphase I laggards than could be accounted for by the lagging of metaphase I univalents. This is in agreement with the results reported previously for autotetraploids (13, 14). In diploid *Lolium perenne*, MYERS (11) found that the frequency of anaphase I laggards was less than expected from the number of metaphase I univalents, indicating that part of the univalents moved to the poles without lagging and dividing equationally. If a similar situation occurs in *Dactylis glomerata*, the excess of anaphase I laggards is even greater than indicated by the data in table 4. Two explanations of the excess of anaphase I laggards are possible: (*a*) while lagging univalents are easily seen at anaphase I they are less easily observed at metaphase I, where they may be obscured by the main group of chromosomes on the equatorial plate; or (*b*) there may be other sources of anaphase I laggards in addition to unpaired chromosomes at metaphase I. DARLINGTON (3) reported that laggards frequently result from the incomplete disjunction of the chromosomes of multivalents. If that is the case, it might be expected that quadrivalent frequency would be positively correlated with the excess of anaphase I laggards over metaphase I univalents in the various plants. Using data from the twelve plants with duplicate determinations, the correlation coefficient between these two characters was −0.306, a nonsignificant value.

The correlation coefficients of percentage of metaphase I sporocytes with univalents and of anaphase I sporocytes with laggards with the percentage of quartets showing micronuclei were highly significant; P = less than 0.01 (4, table VA). These results are in agreement with the hypothesis that the micronuclei result from the loss of daughter univalents of the longitudinally dividing anaphase I laggards either during the first division, second division, or both. The expected number of micronuclei per 100 quartets may be calculated on the basis of this behavior of anaphase I laggards, if it is assumed that none of the daughter univalents is included in the quartet nuclei (table 4). In eighteen of the plants the obtained number was less than expected, suggesting that some of the daughter univalents are included in the quartet nuclei. This is in agreement with earlier conclusions (14).

Summary

1. Chromosomal association and behavior during meiosis was determined from duplicate collections of twelve plants and single collections of eight

plants from open-pollinated populations of *Dactylis glomerata*.

2. The average number of quadrivalents per sporocyte ranged from 2.42 to 4.39 for the twenty plants. The differences among plants and between dates of collection were statistically significant.

3. The percentage of metaphase I sporocytes with univalents varied from 0 to 16.8. P was less than 0.05 and 0.01, respectively, for comparisons of differences among plants and between dates of collection.

4. From 1.7 to 34.0 per cent of the anaphase I sporocytes of the different plants had lagging chromosomes which were dividing equationally. The difference between dates of collection was significant. For comparison of variance due to plants with the interaction plants × days, F was slightly less than F for P of 0.05. From 0.5 to 13.8 per cent of the anaphase I sporocytes had dicentric chromatid bridges accompanied by acentric fragments.

5. Significant differences between the dates of collection and among plants were found for percentage of quartets with micronuclei. The plants ranged from 1.5 to 25.6 per cent.

6. Quadrivalent frequency was not correlated with percentage of metaphase I sporocytes having univalents, percentage of anaphase I sporocytes with laggards, or percentage of quartets with micronuclei. Correlation coefficients were significant between metaphase I sporocytes with univalents and anaphase I with laggards, between metaphase I with univalents and quartets with micronuclei, and between anaphase I with laggards and quartets with micronuclei.

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LITERATURE CITED

1. BLISS, C. I., The analysis of field experimental data expressed in percentages. Plant Protection Bull. 12. U.S.S.R. 1937. (Russian with English summary.)
2. ———, The transformation of percentages for use in the analysis of variance. Ohio Jour. Sci. 38:No. 1. 1938.
3. DARLINGTON, C. D., Chromosome behavior and structural hybridity in the Tradescantiae. Jour. Gen. 21:207–286. 1929.
4. FISHER, R. A., Statistical methods for research workers. Oliver and Boyd, London. Ed. 6. 1936.
5. FISHER, R. A., and YATES, F., Statistical tables for biological, agricultural, and medical research. Oliver and Boyd, London. 1938.
6. MÜNTZING, A., Quadrivalent formation and aneuploidy in *Dactylis glomerata*. Bot. Not. 1933:198–205. 1935.
7. ———, The effects of chromosomal variation in *Dactylis*. Hereditas 23:113–235. 1937.
8. ———, Chromosomenaberrationen bei Pflanzen und ihre genetische Wirkung. Zeitschr. Induk. Abst. Vererb. 76:323–351. 1939.
9. MYERS, W. M., Tetrasomic inheritance in *Dactylis glomerata*. Genetics 25:126. 1940. (Abstract.)
10. ———, Genetical consequences of the chromosomal behavior in orchard grass, *Dactylis glomerata* L. Jour. Amer. Soc. Agron. 33:893–900. 1941.
11. ———, Variations in chromosomal behavior during meiosis among plants of *Lolium perenne* L. Cytologia 11:388–406. 1941.
12. ———, Meiotic behavior of *Phleum pratense*, *Phleum subulatum*, and their F_1 hybrid. Jour. Agr. Res. 63:649–659. 1941.
13. MYERS, W. M., and HILL, HELEN D., The association and behavior of chromosomes in autotetraploid grasses. Genetics 25:129. 1940. (Abstract.)
14. ———, and ———, Studies of chromosomal association and behavior and the occurrence of aneuploidy in autotetraploid grass species, orchard grass, tall oat grass, and crested wheatgrass. BOT. GAZ. 102:236–255. 1940.
15. ———, and ———, Variations in meiotic behavior among plants of the autotetraploid *Dactylis glomerata*. Genetics 26:162. 1941. (Abstract.)
16. ÖSTERGREN, G., Cytology of *Agropyron junceum*, *A. repens*, and their spontaneous hybrids. Hereditas 26:305–316. 1940.
17. RICHARDSON, M. M., Structural hybridity in *Lilium martagon album* × *L. hansonii*. Jour. Gen. 32:411–450. 1936.
18. SNEDECOR, G. W., Calculation and interpretation of analysis of variance and covariance. Collegiate Press, Ames, Iowa. 1934.

INFLUENCE OF CONTROLLED-ATMOSPHERE STORAGE ON RESPIRATION OF McINTOSH APPLES

ROBERT M. SMOCK

(WITH FOUR FIGURES)

Introduction

The fundamentals of the procedure of controlled-atmosphere storage of fruit were developed by KIDD and WEST (2) in England. The application of this method to New York and New England varieties of apples has been described by SMOCK and VAN DOREN (3).

This method seems unusually well adapted to the McIntosh apple. When held in 5 per cent carbon dioxide and 2 per cent oxygen at a temperature of 40° F., this variety keeps two or three times as long as in ordinary cold storage in air at 32° F. (3). Not only do the apples keep firmer and more crisp, but they escape the physiological disorder known as brown core, which often occurs in cold storage at 32° F. with this variety. One of the most striking results is a "residual effect" of the storage treatment after removal from storage. McIntosh apples treated in this manner seem to keep longer than might be anticipated on the basis of their condition upon removal from storage.

This investigation was initiated to study possible differences in respiration rate between McIntosh apples in ordinary cold storage at 32° F. and in controlled-atmosphere storage at 40° F. A possible explanation of the residual effect of this storage technique was also sought.

METHODS.—Uniformly mature McIntosh apples were selected from several moderately vigorous trees grown under identical conditions in the Cornell University orchard at Ithaca, New York. The fruit was slightly more nearly mature when harvested in 1940 than in 1941. Fruit firmness was measured with a Magness-Taylor pressure tester and ground color with a standard apple-color chart.

The respiration rate of apples held in air was measured by passing carbon dioxide-free air over lots of ten fruits each at a rate of 27–28 liters per hour and absorbing the evolved carbon dioxide. The respiration rate of fruit held in controlled atmospheres was measured by the method described by VAN DOREN (4), using lots of 250 apples each. CLAYPOOL'S (1) method for the determination of carbon dioxide in the internal atmospheres of the fruit was used. This method measures more than free, gaseous carbon dioxide but it seemed to give results which were comparable and could be duplicated.

Results

EFFECTS ON LENGTH OF KEEPING

Table 1 gives data on the effect of the two storage treatments on the firmness, changes in ground color, and length of period of marketability of the fruit upon removal from storage. The data show that controlled-atmosphere storage has in general retarded losses in firmness as measured by the pressure test and retarded the change in color from yellowish green to yellow on the fruit. Upon removal in January, the controlled-at-

mosphere fruit remained marketable twice as long as cold-storage fruit. By the first of May, cold-storage apples were either unmarketable upon removal from storage or remained so for only a few days. Controlled-atmosphere apples remained marketable for 3–4 weeks after removal from storage in May.

TABLE 1

INFLUENCE OF STORAGE TREATMENT ON KEEPING QUALITY OF McINTOSH APPLES (A, STORED AT 32° F. IN AIR; B, STORED IN 5% CO_2–2% O_2 AT 40° F.)

DATE	PRESSURE TEST (LB.)		GROUND COLOR (UNITS)		PERIOD OF MARKETABILITY AFTER REMOVAL FROM STORAGE (DAYS)	
	A	B	A	B	A	B
	1940–41 SEASON					
Oct. 7, 1940 (harvest)	12.4	12.4	3	3	52 (not stored)	.....
Jan. 1, 1941	10.1	10.8	3–4	3–4	32	43
Mar. 1, 1941	9.4	11.5	3–4	3–4	11	30
May 1, 1941	7.2	9.6	3–4	3–4	0	26
	1941–42 SEASON					
Sept. 19, 1941 (harvest)	16.0	16.0	2½–3	2½–3	.....	.....
Jan. 2, 1942	10.5	15.4	2½–3	2½–3	24	42
Mar. 3, 1942	9.2	13.7	3–4	2½–3	12	35
May 4, 1942	10.5	13.1	4	3	3	31

EFFECTS ON RESPIRATION RATE

SEASON 1940–41 DATA.—Figure 1 shows results obtained on the respiration rate of apples held in 32° F. air and 5 per cent carbon dioxide and 2 per cent oxygen at 40° F. It was not known definitely whether the fruit was picked in a pre-climacteric or post-climacteric condition.[1] On the basis of respiration data obtained, they were pre-climacteric. The respiration rate during the storage period was approximately two or three times as fast at 32° F. in air as it was in controlled-atmosphere storage at 40° F. (fig. 1). A clear-cut respiratory rise culminating in a climacteric was not noted in the cold-storage lot, although there was a suggestion of such a rise during December. It seems safe to conclude that there was no respiratory rise in the controlled-atmosphere apples while in storage.

When the respiration rate of the fruit was measured upon removal from storage to air at 66° F. in January, the cold-storage apples were declining in respiratory rate and were apparently post-climacteric. The controlled-atmosphere apples went through a normal respiratory rise, reached a respiratory peak, and declined. This would confirm the as-

[1] Following harvest, apples and certain other fruits go through a respiratory rise until they reach a peak, called the "climacteric" by KIDD and WEST (2). The climacteric is followed by a more or less gradual decline in respiratory intensity.

sumption that the apples were pre-climacteric when picked and that they did not experience a respiratory rise during storage. The most significant observation on these data, however, is that the latter did not respire so fast as the fruit was near the end of its marketability. The sudden rise after the first day's measurement was due to the fact that the fruit began to split open. The controlled-atmosphere fruit again went through a normal respiratory curve.

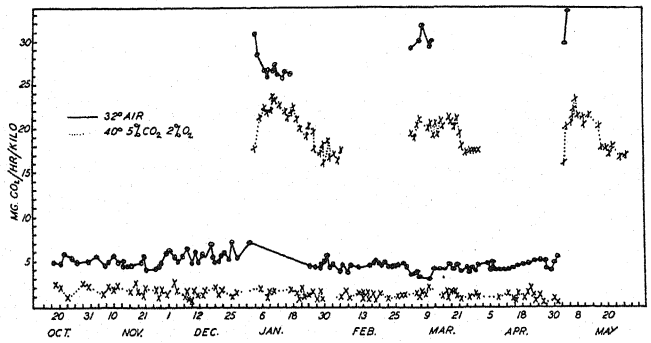


FIG. 1.—Respiration of McIntosh apples during 1940–41 storage season in 32° F. air and in controlled-atmosphere storage at 40° F. In January, March, and May, samples removed from these two storage treatments and respiration measured in air at 66° F. (upper set of curves).

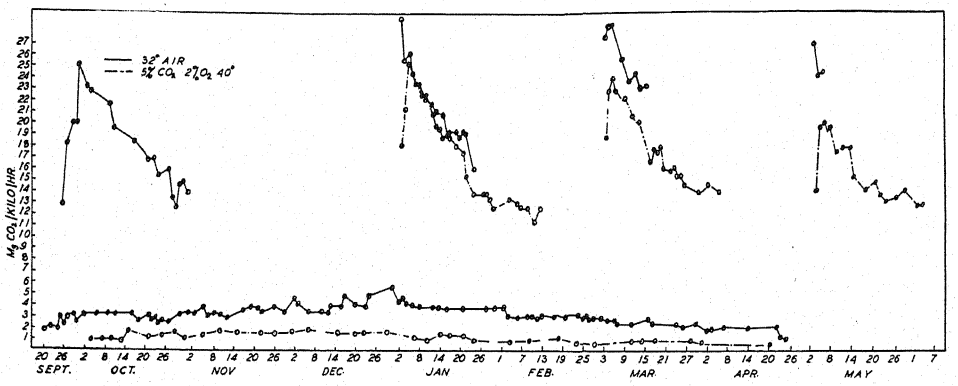


FIG. 2.—Respiration of McIntosh apples during 1941–42 storage season in 32° F. air and controlled-atmosphere storage at 40° F. Respiration also measured (upper set of curves) on fruits at harvest time and upon removal from storage in January, March, and May at 74° F.

mer, even though held under identical conditions upon removal. The controlled-atmosphere fruit upon removal from storage in March went through a normal respiratory curve and at no time respired as fast as the cold-storage fruit.

When the respiration rates were measured upon removal from storage in May, it was apparent that the cold-storage

SEASON 1941–42 DATA.—The respiration rate of the fruit at harvest time was measured, as well as after removal from storage treatments at different dates during this season. The respiration curve (fig. 2) at harvest time shows the fruit definitely pre-climacteric at the time of picking.

Respiration data during storage indi-

cate that the cold-storage (32° F. air) fruit respired approximately two to three times as fast as the controlled-atmosphere fruit. The former seemed to reach a respiratory peak in late December. The only semblance of a respiratory peak during the previous season came at this same period. The controlled-atmosphere fruit seemed to have no respiratory rise while in storage. The fact that it did have such a rise when removed from storage tends to confirm this fact, since apples seldom have two such rises.

When apples were removed from cold storage in January, they declined in their respiration rate at 74° F., indicating that they had passed their respiratory peak in storage. The controlled-atmosphere apples went through a normal respiratory curve when removed from storage. Upon removal in March, the cold-storage apples again gave indication of being post-climacteric. Again the controlled-atmosphere apples went through a normal respiratory curve, and at no time did they respire as rapidly as the cold-storage apples.

Upon removal from storage in May, the cold-storage apples seemed to be at about the end of their respiratory curve, becoming unmarketable after 3 days at 74° F. The controlled-atmosphere apples went through their normal respiratory curve and respired considerably less than the cold-storage apples.

The longer the controlled-atmosphere apples were held in storage, the slower they respired upon removal. This fact was more apparent during the 1941–42 season than during the previous one. No great significance can be attached to this observation, however, since it should be remembered that the apples were also becoming older with time, in spite of the intensity of their respiration rate.

After removal from controlled atmosphere in May, 1942, one lot of McIntosh apples was moved into air storage at 32° F. Although the data are not presented here, the respiration rate of these apples was about halfway between that of apples held in 32° F. air continuously and those held in controlled atmospheres at 40° F. In other words, there was a residual effect on these controlled-atmosphere apples following their removal to ordinary cold storage at 32° F. These apples started their climacteric rise after 18 days at 32° F.

Cumulative respiration results

Season 1940–41 data.—From the smoothed respiration curves, cumulative curves of the respiration rate were constructed for the two storage treatments and plotted at 3-day intervals. By the first of January the cold-storage apples had evolved about three times as much carbon dioxide as the controlled-atmosphere fruit (fig. 3). Upon removal to a temperature of 66° F. in air, it required twice as long for the controlled-atmosphere apples to become unmarketable and to evolve the same amount of carbon dioxide as the cold-storage apples when discarded.

By March the cold-storage fruits had given off more than three times as much total carbon dioxide as the controlled-atmosphere fruit. Upon removal to 66° F. air, the controlled-atmosphere apples remained marketable three times as long as the cold-storage apples. Upon removal in May, the cold-storage apples were unmarketable and had given off more total carbon dioxide than the other lots. The cold-storage apples had given off three times as much carbon dioxide as the controlled-atmosphere fruits and were unmarketable upon removal.

With one exception, all lots of McIntosh apples during this year's trial

seemed to become unmarketable when they had given off 18–19 gm. of carbon dioxide per kilo of fruit. The cold-storage apples upon removal in March reached a considerably higher figure than this.

fruit seemed to become unmarketable when it evolved a total of 17.5–19.5 gm. of carbon dioxide per kilo of fruit.

The period of marketability was not measured on the fruit held at 74° F. fol-

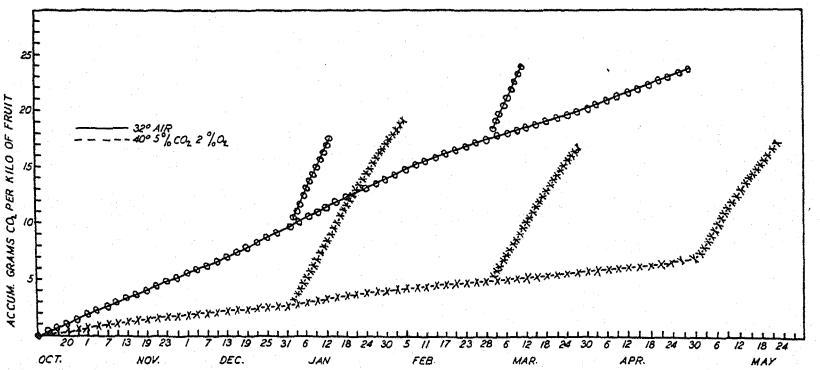


FIG. 3.—Cumulative respiration of McIntosh apples during 1940–41 season in 32° F. air and controlled-atmosphere storage at 40° F. (the two most nearly horizontal lines). In January, March, and May, samples removed from these two storage treatments and respiration measured in air at 66° F. (the five most nearly vertical lines).

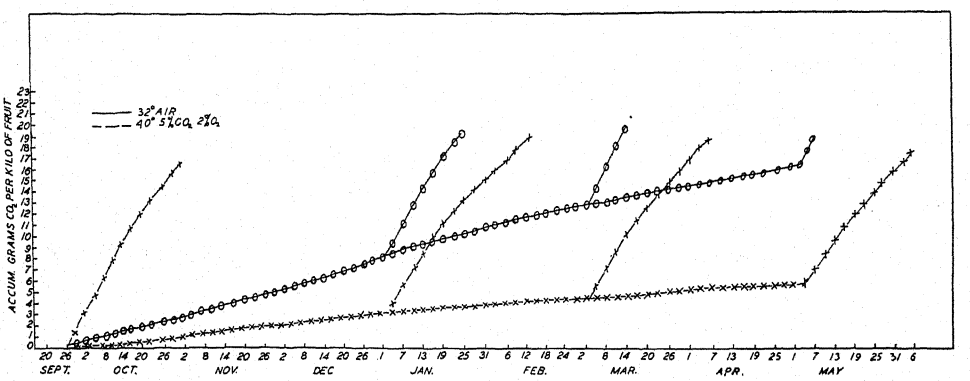


FIG. 4.—Cumulative respiration of McIntosh apples during 1941–42 season in 32° F. air and controlled-atmosphere storage at 40° F. (the two most nearly horizontal lines). Cumulative respiration at harvest time and upon removal from storage in January, March, and May at 74° F. (the seven most nearly vertical lines).

SEASON 1941–42 DATA.—By the first of January the cold-storage apples had given off a total of almost two and one-half times as much carbon dioxide as the controlled-atmosphere fruit (fig. 4). By the first of March and May, the cold-storage fruit had given off about three times as much carbon dioxide as the controlled-atmosphere fruit. During this season the lowing harvest, so the high point of the cumulative curve for this lot of fruit should not be included in comparisons.

INTERNAL ATMOSPHERE OF FRUIT

One explanation of the "residual effect" of controlled atmosphere storage treatment is the possible persistence of carbon dioxide within the tissues of the

fruit. An equally plausible explanation would be the persistence of a low oxygen atmosphere within the fruit upon removal from storage. This latter point was not tested.

Table 2 gives the results of analyses of carbon dioxide in the internal atmospheres of the fruit. Storage in 5 per cent carbon dioxide resulted in a considerably higher carbon dioxide content in the fruit immediately upon removal from storage. Of course, the air-storage sample was at a lower temperature (32° F.), but respiration was slower at the higher temperature. The 32° F. air lot had a greater temperature differential between fruit and room temperature than the controlled-atmosphere lot. On March 22, 1941, the controlled-atmosphere lot had approximately four times as much carbon dioxide in its tissues as had the cold-storage lot immediately upon removal. Within $2\frac{1}{2}$ hours the carbon dioxide in the controlled-atmosphere fruits had dropped almost 100 per cent. A level of 117 mg. of carbon dioxide per kilo of fruit was not reached in the 32° F. air lot until after 48 hours. Fruit temperatures had reached air temperatures within 24 hours, however. These analyses were repeated on similar apples in April and similar results were obtained, although it took the controlled-atmosphere apples 4 hours to reach the carbon dioxide level which they later maintained—at a rather constant figure.

From these data it would seem that the residual effect of controlled-atmosphere treatment of apples cannot be explained on a basis of persistence of carbon dioxide in the internal atmospheres of the fruit. Diffusion of this gas was somewhat rapid under the conditions of the experiment. Diffusion might have been less rapid in apples confined in a container than in these that were lying on a laboratory table. Oxygen data were not obtained.

TABLE 2

CARBON DIOXIDE IN INTERNAL ATMOSPHERE OF McINTOSH APPLES FOLLOWING REMOVAL FROM STORAGE TO ROOM TEMPERATURE AS AFFECTED BY STORAGE TREATMENT

Treatment	Hours after removal	Mg. CO_2 per kilo of fruit	Treatment	Hours after removal	Mg. CO_2 per kilo of fruit
5% CO_2 and 2% O_2 at 40° F.	0	209	32° F. air	0	57
5% CO_2 and 2% O_2 at 40° F.	$2\frac{1}{2}$	117	32° F. air	$2\frac{1}{2}$	57
5% CO_2 and 2% O_2 at 40° F.	$4\frac{1}{2}$	110	32° F. air	$4\frac{1}{2}$	88
5% CO_2 and 2% O_2 at 40° F.	48	113	32° F. air	48	124

Discussion

It seems rather evident from these results with McIntosh apples that the use of controlled-atmosphere storage (5 per cent carbon dioxide and 2 per cent oxygen at 40° F.) reduced the respiration rate in storage about 66 per cent in comparison with apples in 32° F. air. When removed from storage in controlled atmospheres McIntosh apples do not respire as fast as fruit removed from cold storage. This may partially explain the so-called "residual effect" of controlled atmospheres. Use of such atmospheres might have affected respiratory enzymes. VAN DOREN (4) has found that controlled atmospheres also seem to affect the enzymes involved in pectose hydrolysis in

apples. There may be still other effects, the sum total of which would explain this residual effect of storage. Persistence of high carbon dioxide atmospheres within the fruit after removal from storage would hardly explain this effect, since no such persistence could be found. While oxygen levels within the fruit were not measured, it seems unlikely that this would offer an explanation.

Cumulative respiration curves indicate that cold-storage fruit evolved a sum total of two and one-half to three times as much carbon dioxide as controlled-atmosphere fruit within the same time period in storage.

The use of cumulative respiration curves as an index of the "length of life" of the fruit has certain values and certain limitations. During the 1940–41 season, the McIntosh apples seemed to become unmarketable when they had evolved a total of 18–19 gm. of carbon dioxide per kilo of fruit. During the following season they evolved 17.5–19.5 gm. before becoming unmarketable. The fruit was picked in a slightly less mature condition in the latter season, however. The possibility of using such a figure as 18 or 19 gm. of carbon dioxide per kilo of fruit as a physiological expression of the length of marketable life of McIntosh as grown in a given selection should be tested further. It is apparent that to use such an expression the fruit would have to be examined from one production area only, using fruit picked at the same point on the respiration curve each year and avoiding such secondary factors as rotting, scald, and internal disorders. Some of the complicating factors in this study were brown core (a low temperature disorder) and a type of arsenical injury which appeared on the calyx end of the fruit when it was held in a high relative humidity for extended periods. In spite of the numerous limitations of this expression, it deserves further testing.

Summary

1. McIntosh apples respired approximately one-third as fast in controlled-atmosphere storage at 40° F. as in ordinary cold storage at 32° F. The residual effect of controlled-atmosphere storage on apples after their removal to high temperatures can be partially explained by a reduction in respiration rate following the storage treatment. The residual effect cannot be accounted for by persistence of a high carbon dioxide level in the tissues of the fruit.

2. McIntosh held in cold storage at 32° F. evolved two and one-half to three times as much carbon dioxide during the same time period in storage as controlled-atmosphere fruit. The value and limitations of cumulative respiration curves for this type of study are indicated.

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LITERATURE CITED

1. CLAYPOOL, L., Internal gas in fruits as influenced by external treatments. I. Carbon dioxide. Proc. Amer. Soc. Hort. Sci. 36:374–378. 1939.
2. KIDD, F., and WEST, C., Refrigerated gas storage of apples. Food Investigations Bd. (Gt. Britain) Leaflet 9. 1936.
3. SMOCK, R. M., and VAN DOREN, A., Controlled atmosphere storage of apples. Cornell Agr. Exp. Sta. Bull. 762. 1941.
4. VAN DOREN, A., The influence of controlled atmospheres on the storage life and keeping qualities of certain varieties of apple fruits. Cornell Univ. Ph D. Thesis. 1941.

DEVELOPMENT OF THE MACROGAMETOPHYTE OF MIERSIA CHILENSIS

MARION S. CAVE

(WITH TEN FIGURES)

Introduction

Miersia is a South American genus belonging to the tribe Gilliesieae. This tribe, together with the closely related Agapantheae and Allieae, has been transferred by HUTCHINSON (3) from the Liliaceae to the Amaryllidaceae on the basis of the umbellate inflorescence with an involucre of bracts. In the Agapantheae, *Tulbaghia violacea* follows the *Scilla* type of development of the female gametophyte, whereas *Agapanthus umbellatus* follows the "normal" type (9). In the Allieae, WEBER (13) and MESSERI (7) have shown that a great number of *Allium* species belong to the *Scilla* type. This is also the case in *Allium cepa* (4), *A. govanianum* (12), and *A. fistulosum* (11). *Nothoscordum fragrans*, according to STENAR (10) and MESSERI (7), is like *Scilla*, but *N. striatum* is normal, although MESSERI finds occasional cases where *N. striatum* follows the *Scilla* type. In *Nothoscordum bivalve* ECKLES (2) states that the development is normal. *Leucocoryne ixioides* (1) is like *Scilla*.

Since some bulbs of *Miersia chilensis* were received at the Botanic Garden of the University of California from Valparaiso, Chile, an opportunity was provided to investigate macrosporogenesis and macrogametophyte development in a member of the tribe Gilliesieae, in which no studies have been made so far as the writer is aware.

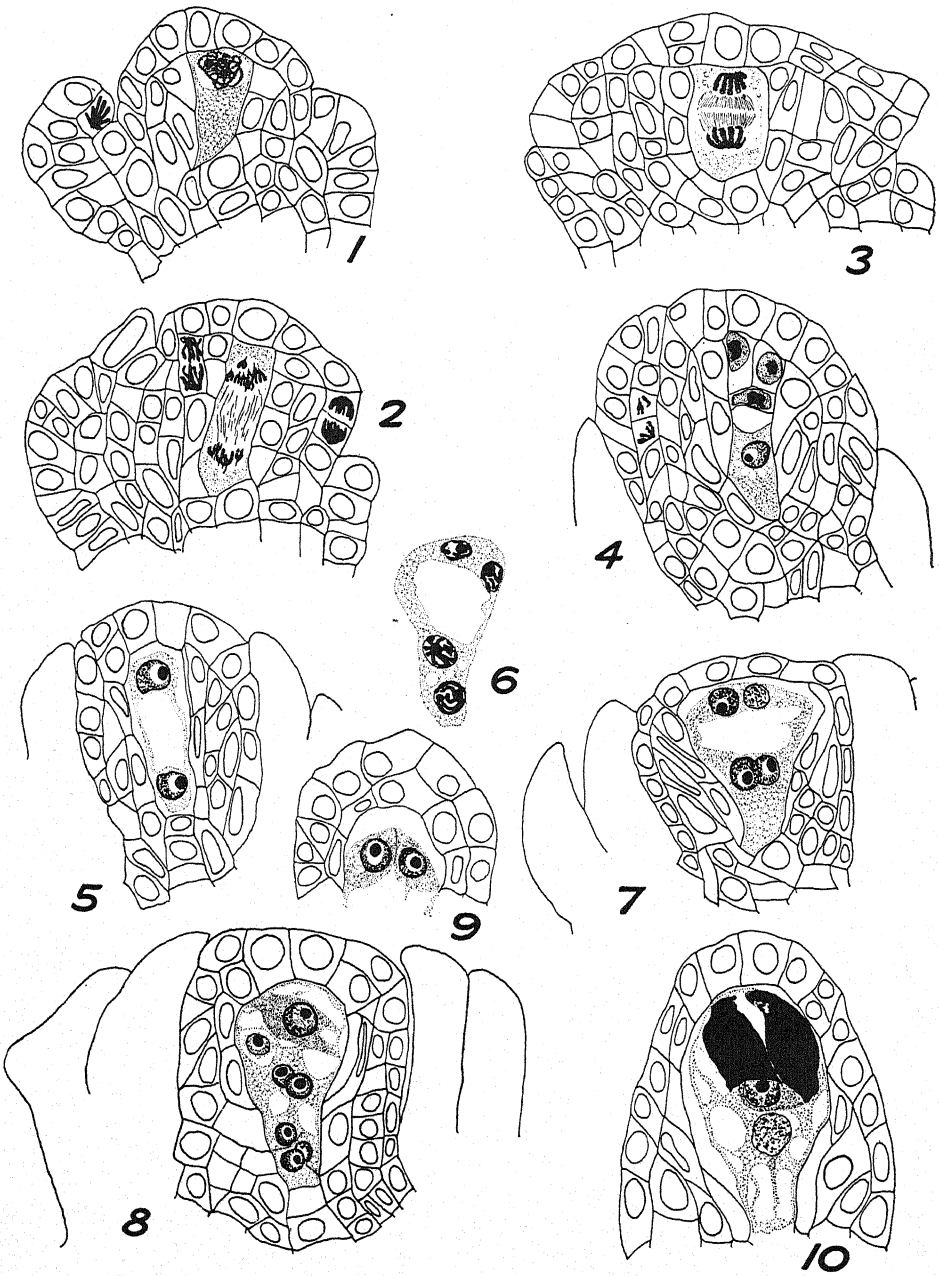
Investigation

The bulbs were planted in September, and plants were grown both in the greenhouse and outdoors in Berkeley. They flower from November through February. Each umbellate inflorescence has from three to six flowers, all at different stages of growth. Ovaries of one inflorescence fixed at the time the anthers of the oldest flower are shedding their pollen show a succession of stages in development of the female gametophyte, from prophase of the archesporial cell through the mature macrogametophyte. All ovaries were fixed in CRAF, dehydrated in butyl alcohol, and stained in iron-alum haematoxylin. Sections were cut at 10 μ.

The archesporial cell (fig. 1) is situated directly beneath a layer of nucellus cells. The extremely small number of the latter is striking. In cross-section, the entire length of the archesporial cell has only about two nucellar cells at each side of it.

Meiosis proceeds normally (figs. 2, 3). The formation of macrospores always results in a T-shaped configuration (fig. 4). The chalazal macrospore grows considerably in size before starting division. At this time the inner integument has not yet surrounded the nucellus and the outer integument shows a slight bulge on one side of the ovule.

The divisions of the nucleus of the enlarged chalazal macrospore then take place in rapid succession. At the two-nucleate stage the nuclei lie at opposite ends of the developing macrogametophyte, separated by a vacuole (fig. 5). At the next division the micropylar nucleus always divides at right angles to the



FIGS. 1–10.— Macrosporogenesis and development of gametophyte in *Miersia chilensis:* Fig. 1, archesporial cell in prophase. Fig. 2, anaphase of first meiotic division. Fig. 3, telophase of first meiotic division. Fig. 4, four macrospores with micropylar three degenerating. Fig. 5, two-nucleate gametophyte. Figs. 6, 7, four-nucleate gametophyte. Fig. 8, eight-nucleate gametophyte; one synergid not shown. Fig. 9, synergids before degeneration. Fig. 10, two degenerated synergids, egg nucleus, and part of primary endosperm nucleus. ×350.

long axis of the macrogametophyte, while the nucleus at the chalazal end may divide likewise, or parallel with the long axis (figs. 6, 7). The four nuclei then divide to produce eight, and a polar nucleus from each group of four migrates to the center (fig. 8). These fuse to produce the primary endosperm nucleus. The antipodals do not degenerate, and at the micropylar end of the macrogametophyte a typical egg cell with a large vacuole is formed (fig. 10). The synergids grow to a large size and then seem to degenerate, at which time they stain deeply with haematoxylin (figs. 9, 10).

The integuments do not entirely surround the nucellus, even when the macrogametophyte is mature, and the small number of cells which make up the nucellus and integuments is very noticeable.

According to SCHNARF (8) and MAHESHWARI (5, 6), the origin and mode of development of the macrogametophyte should be one line of evidence in working out the phylogeny of groups in the angiosperms. In *Miersia chilensis*, the first member of the Gilliesieae studied, the type of development is "normal." In the two closely related tribes both the normal and *Scilla* types are found. In *Allium* all the species so far investigated belong to the *Scilla* type, whereas within the genus *Nothoscordum* both schemes of development occur. At present too small a number of species has been studied to be of much aid in working out phylogeny, but further work along this line should give an insight into the relationships existing both within and among these three closely related tribes.

Summary

1. Macrosporogenesis and development of the macrogametophyte in *Miersia chilensis* is "normal."

2. Investigation into the origin and mode of development of the macrogametophyte in other species of the Agapantheae, Allieae, and Gilliesieae is necessary before this character can be used in working out the phylogenetic relationships existing within and among these tribes.

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LITERATURE CITED

1. CAVE, M. S., Macrosporogenesis in *Leucocoryne ixioides* Lindl. Cytologia 9:407–411. 1939.
2. ECKLES, M. L., Megasporogenesis and development of the megagametophyte in *Nothoscordum bivalve*. BOT. GAZ. 102:806–809. 1941.
3. HUTCHINSON, J., The families of flowering plants. Vol. II: Monocotyledons. 1934.
4. JONES, H. A., and EMSWELLER, S. L., Development of the flower and macrogametophyte of *Allium cepa*. Hilgardia 10:415–428. 1936.
5. MAHESHWARI, P., A critical review of the types of embryo sacs in angiosperms. New Phytol. 36: 359–417. 1937.
6. ———, Recent work on the types of embryo sacs in angiosperms: a critical review. Jour. Ind. Bot. Soc. 20:229–261. 1941.
7. MESSERI, A., Recerche embriologiche e cariologiche sopra i generi *Allium* e *Nothoscordum*. Nuovo Gior. Bot. Ital. N.S. 38:409–441. 1931.
8. SCHNARF, K., Contemporary understanding of embryo-sac development among angiosperms. Bot. Rev. 2:565–585. 1936.
9. STENAR, H., Zur Embryologie der *Agapanthus*-Gruppe. Bot. Not. 1:520–530. 1933.
10. ———, Studien über die Entwicklungsgeschichte von *Nothoscordum fragrans* Kunth. und *N. striatum* Kunth. Svensk. Bot. Tid. 26:25–44. 1932.
11. STRASBURGER, E., Die Angiospermen und die Gymnospermen. Jena. 1879.
12. SUNDARA RAO Y., Structure and development of the embryo-sac of *Drimiopsis kirki* Baker and *Allium govanianum* Wall. Jour. Ind. Bot. Soc. 19:273–282. 1940.
13. WEBER, E., Entwicklungsgeschichtliche Untersuchungen über die Gattung *Allium*. Bot. Archiv. 25:1–44. 1929.

CURRENT LITERATURE

Deciduous Orchards. By WILLIAM HENRY CHANDLER. Philadelphia: Lea & Febiger, 1942. Pp. 438. $4.50.

This volume adds to the discussion of the usual list of deciduous fruit trees such others as mulberry, fig, persimmon, papaw, pomegranate, jujube, walnut, hickory, hazelnut, chestnut, pistachio, and tung. Although the treatment of some of these is brief, there is at least an establishment of their place in American horticultural textbooks.

In several early chapters, tree and fruit structure, processes, responses, and relationships are discussed. These are correlated with the setting and development of fruits and the processes which may occur in mature fruits. The whole treatment resolves itself into an effective ecological treatise on the culture of fruit trees. The reader is stimulated to initiate additional experiments to aid in extending knowledge on nearly every topic presented. Every teacher will be interested in the author's ideas on teaching as presented in the preface.—E. J. KRAUS.

Chemistry and Physiology of the Vitamins. By H. R. ROSENBERG. Interscience Publishers, Inc., 1942. Pp. xix+674. Illustrated. $12.00.

This is an important monograph, summarizing the current knowledge of the chemical structure of the known vitamins and their physiological functions. The first chapter is a general survey, concerned with definitions, historical information, nomenclature, lists of identified and unidentified vitamin entities, occurrence, isolation, constitution of molecules, syntheses, methods of preparation, biogenesis, specificity, determination, standards, the general physiology of vitamins, and pathological problems such as avitaminoses, hypovitaminoses, hypervitaminoses, and normal requirements.

Then the vitamins are taken up in alphabetical order. The vitamins A come first, with consideration of the provitamins of this group, followed by sections on vitamin A_1, A_2, A_3—their biogenesis, determination, physiological importance, pathological implications, and requirements.

Several chapters are devoted to the B group—B_1, B_2, and B_6. The ground covered for each vitamin follows the same general pattern in each case, a thorough assembling of what has been established of the chemistry and physiology of each. Other chapters deal with nicotinic acid and nicotinamide; pantothenic acid; inositol; and para-amino-benzoic acid; members of the B complex. Ascorbic acid (vitamin C), the group of vitamins D, the group of vitamins E, biotin (vitamin H), the group of vitamins K, vitamin P, and a group of nonidentified vitamins complete the main body of the work. An appendix deals with so-called "vitagens," such as essential fatty acids, essential amino acids, essential carbohydrates, choline, and transferable methyl groups.

The nonidentified vitamins contain a heterogeneous group of B complex substances: vitamins J, L_1 and L_2, and M, factor T, factor U, folic acid, and such problematical substances as grass juice factor, mouse factor, and antipernicious anemia factor.

One interesting feature of the book is a patent index, which occupies almost 60 pages. The list includes foreign patents as well as those issued in the United States. The patents cover methods of extraction, preservation of vitamin content in foods, concoction of elixirs, apparatus concerned with the manufacture of preparations, additions of vitamins to food products, tablets for medical use, and many other features of commercial vitamin production and consumption.

The author index, three columns to the page, fills 44 pages. A well-chosen subject index completes this useful volume.

The author of the work is a vitamin chemist in the Jackson Laboratory of the E. I. Du Pont de Nemours and Company; he has had this volume in mind for almost 15 years. His work with Ruzicka and Reichstein in Switzerland at about the time the first vitamin was becoming structurally known was the stimulus which finally brought the work into existence. It is a very creditable survey, and as a source book of knowledge it should be of great value to biochemists and plant physiologists.—C. A. SHULL.

A Short History of the Plant Sciences. By HOWARD S. REED. Waltham, Mass.: Chronica Botanica Co.; New York: G. E. Stechert & Co., 1942. Pp. x+323. Figs. 37. $5.00.

This book "is intended for the average graduate student,—to guide students during the formative period to a better understanding of past and present trends, and to clarify the increasingly complex situation into which they will eventually be thrust." This purpose the volume will accomplish only in part, although most students (and their teachers) will find much of interest and value in it. No one writing on such a subject could escape criticism, unless he first apologized, as Professor REED does, for the book's reflection of his own interests and for its omissions and inability to treat certain subjects adequately. Yet perhaps a better title would have been "Topics in the history of the plant sciences."

The first third of the treatment is unified, and most phases of plant science are covered up to the

beginning of the nineteenth century, often including material not easily available. This section is concluded with a chapter on gardens, in which the author brings out the influence of gardens and gardening on the development of the plant sciences. From here on he has deliberately omitted, he states, any discussion of systematic botany, phylogeny, paleobotany, genetics and plant breeding, because of adequate treatment of them in recent books. For the same reason he has chosen not to discuss the new work on growth, tropisms, and hormones, although there is little or no mention of any of the work on growth, irritability, respiration, photoperiodism, and the physiology of reproduction during either the nineteenth or twentieth centuries. Ecology and plant geography are treated only up until 1900, since the author feels unable to discuss the more recent concepts, which—to him—lack precise definition.

With these omissions, it was natural to compartmentalize the topics covered. The chapters on morphology, cytology, mycology, and plant pathology could be used in conjunction with textbooks in the respective fields, especially the last three. Physiology is covered at much greater length in six chapters on water economy, carbon fixation, nitrogen assimilation, fixation, and metabolism of nitrogen, plant nutrition, and mineral constituents in metabolism. Although the great development in certain fields of plant science in the last century and a half of increasing specialization is thus emphasized, one wonders just how the book may be used to best advantage. For the teacher the heuristic approach is of value only as it emphasizes the mutual dependence of the various fields in their development. As a textbook for a course in the history of botany, the incompleteness, and in part the organization, necessarily detract from the value of the volume. For the student, reading independently and capable of integrating his own facts, it would seem desirable that a revision might include a list of suggested references covering adequately, so far as possible, the fields omitted in this edition.—C. E. OLMSTED.

Geographical Guide to Floras of the World. Part I. By S. F. BLAKE and ALICE C. ATWOOD. Washington, D.C.: U. S. Government Printing Office; U.S. Department of Agriculture Miscellaneous Publication no. 401, 1942. Pp. 336. $0.75.

This bibliography is "an annotated catalog of all the now useful floras and floristic works . . . . that list or describe the complete vascular flora (or the phanerogams only) of any region or locality," and of "all publications dealing on the same scale with useful and medicinal plants, vernacular names, and botanical bibliography," complete through 1939. "Publications dealing with only a part of the flora . . . . are excluded, except for complete papers on edible, medicinal, or woody plants." The annotations are brief descriptions of the contents of the listed works, but no attempt was made to evaluate them. Lists covering exceedingly small areas, and those very incomplete or full of errors, have been omitted.

Part I covers the entire world, except Europe, Asia, and the islands near them. The titles included number about 3000 and were selected from more than twice that number of works examined. These are grouped under 229 alphabetically arranged geographical subdivisions in five major regions: Africa, Australasia, Insular, North America, and South America. Lists for the major regions require 48, 24, 44, 102, and 31 pages, respectively. General and local works are listed separately under each subdivision.

The introductory section provides data for summing up our detailed knowledge, or lack of it, of the floras of the portions of the world covered. Other features adding to the usefulness of the book are a list of 100 floras and floristic works of greatest general utility, a complete list of abbreviations for publications cited, and very complete author and geographical indices, the former with authors' dates. —C. E. OLMSTED.

Bibliography of References to the Literature on the Minor Elements and Their Relation to Plant and Animal Nutrition. 3d supplement to 3d edition. New York: Chilean Nitrate Educational Bureau, Inc., 1942. Pp. 78.

This supplement follows the pattern of the previous ones. The abstracts are arranged by elements, alphabetically, from aluminum to zinc. Forty-seven elements are included in the index. At the close of the survey by elements three short sections include a few papers under Nutrient solutions, Nutrient elements, and Analyses. The botanical index lists by common names approximately 100 species upon which nutritional investigations have been made during the past year. More than 700 authors are cited and abstracted.

The publication of this supplement each year is a most commendable service, appreciated by agronomists, plant physiologists, and others interested in the rapidly advancing field of plant nutrition.—C. A. SHULL.

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POTASSIUM AND PHOSPHATE NUTRITION OF PINEAPPLE IN RELATION TO NITRATE AND CARBOHYDRATE RESERVES

G. T. NIGHTINGALE

(WITH TWENTY-FIVE FIGURES)

Introduction

The nitrogen requirements of the pineapple plant, for maximum production of fruit, have been found to vary as much as 75 per cent in successive years in essentially the same site. That the quantity of nitrogen needed may vary greatly in different areas, and in the same field in different years, is due in large part to variations in sunshine, temperature, and rainfall. Nevertheless, by methods already described there may be obtained from the growing plant in the field quantitative records that supply the information necessary for efficient use of fertilizer, regardless of location and weather. That these records are not limited in their use only to a single location or season is due to the fact that the concentration of starch in the plant is employed as an index of the net availability of CO_2 (37). Experiments were conducted to determine whether it might be of any practical importance to maintain a balanced relation between nutrients commonly added to the soil as fertilizers and the product of CO_2 nutrition. Following principles already emphasized (25, 35, 36), the nitrogen nutrition of pineapple was first considered in relation to nitrate and carbohydrate reserves (37). A very low content of nitrate was found adequate for greatest possible yields of fruit when carbohydrates were low; when starch reserves were high, maximum production was not obtained unless the plants were essentially filled to capacity with nitrate (fig. 1).

As in the case of some other monocotyledons (57, 58, 59), fertilizer applications made after blossom buds developed were without measurable influence upon yields. But about a year usually elapses from the time pineapple slips are set in the soil until blossom buds appear. During earlier vegetative stages considerable latitude may be permitted in nitrate nutrition and yet only small but significant losses in yield of fruit result. This is because a pineapple plant, low in nitrate in relation to carbohydrates, will absorb and reduce nitrate with extraordinary rapidity when a more liberal external supply of nitrate is made available. The pe-

riod of nitrate deficiency and carbohydrate accumulation, if not prolonged, is thus often balanced by the subsequent interval of new protein synthesis from nitrate, which is far more rapid than that in comparable plants lower in carbohydrates and continuously supplied with abundant nitrate.

Plants still more deficient in carbohydrates in relation to nitrate absorb and reduce nitrate even more slowly. Thus with limited nitrate reduction there is necessarily a corresponding decrease in oxidation rate of carbohydrates or their derivatives (36). Ultimately the plants thus tend to counteract the condition of carbohydrate deficiency, if subsequent weather conditions become favorable for CO_2 assimilation. These responses, however, are not peculiar to pineapple plants (36). Results in harmony with them have recently been reported by HOAGLAND and ARNON (23). For the reasons mentioned, pineapple plants in the earlier stages of vegetative growth can thus tolerate a short interval of moderate nitrate or carbohydrate deficiency without serious effect on production of fruit. But after blossom buds appear there is no further opportunity to influence nitrate reserves. Just prior to and during blossom-bud differentiation, it is essential that the nitrate content of the plant be at the proper level in relation to carbohydrates, or serious losses in yield will occur (37). The concentration of nitrate required in the plant for maximum fruit production in different locations or seasons, even at the critical period of differentiation, may vary by more than 100 per cent. It is proposed to show in this paper that potassium and phosphorus requirements are intimately associated with the required level of nitrate nutrition.

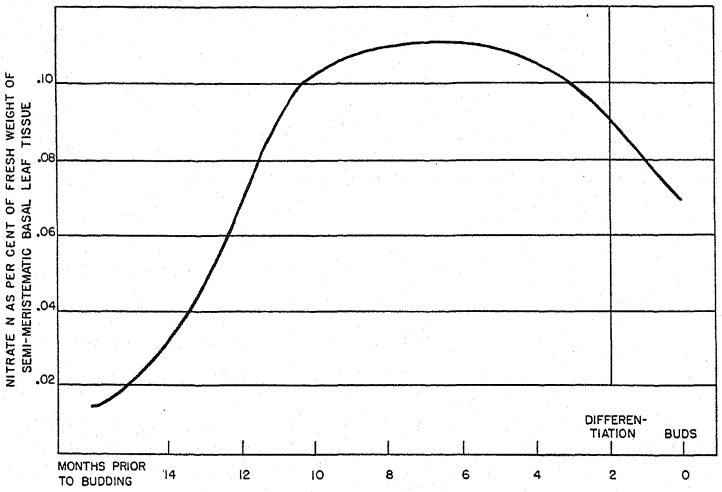


FIG. 1.—Proximate maximum percentages of leaf-nitrate N attainable at different stages of growth. Maximum nitrate is desirable only if plants contain adequate carbohydrate reserves, as indicated in warm sunny location by not less than about 15 per cent and in cool or cloudy location by about 30 per cent no. 1 yellow-green leaf color (37).

Methods

The methods employed have already been described in detail (37). The semimeristematic white basal tissues of pineapple leaves which are nearly fully ex-

panded are employed for nitrate, total potassium, and total phosphorus analyses. The tissues analyzed are comparatively homogeneous and practically free of lignified elements. They consist essentially of young parenchymatous cells, so that for all practical purposes the analytical sample is made up largely of protoplasm. The moisture content of the white basal leaf tissue fluctuates little, even though there may be a severe water deficit in specialized water-storage tissue of the more mature portion of the leaf. Regardless of environmental influences or whether the plants have been growing for 3 months or a year, exactly comparable leaves are always selected for analysis. The tissue analyzed is always semimeristematic and always from leaves of the same stage of development (37). These factors provide a greater degree of precision in interpretation of analytical results than do highly differentiated tissues with varying degrees of lignification and water content. Chemical analyses, particularly for phosphorus, appear to be significant in relation to yields, even when there are only small differences in content of this element in the white basal tissue of the leaves analyzed (37). Great differences in chemical composition among the plants under investigation would scarcely be expected, because a difference in yield of 1 ton of fruit per acre means only 0.125 pound per individual fruit when there are 16,000 plants per acre.

Although not directly comparable with the technique here employed, there is much to be said in favor of analyses of mature tissues (5, 7, 9, 51, 52, 53), particularly where only proximate rapid chemical methods are employed to detect the nutrient deficiencies. Relatively mobile elements, such as nitrogen (38) and potassium (36), commonly become depleted first in the older stem and leaf tissues, because of translocation from mature to meristematic cells. In consequence, lack of these nutrients is often most readily detected in older leaf or stem tissues; and if results are expressed as concentrations found in the sap or in an extract, variations in degree of lignification between unlike samples may perhaps be unimportant.

NITRATE AND CARBOHYDRATE STANDARDS.—The comparatively immature white basal leaf tissue employed for nitrate, potassium, and phosphorus analyses furnishes an excellent index of the content of these nutrients in the expressed juice of the massive stem of the plant. But starch, the major carbohydrate reserve of the vegetative organs, is not stored in quantity in the very young basal leaf tissues. Macroanalyses of the stem and of the mature portions of the leaf do not indicate to what percentage of capacity the plants may be filled with starch. This is due to the fact that relatively xeromorphic yellow-green plants often contain such a high proportion of inert lignified elements that, even though essentially all nonsenescent starch-storing parenchymatous cells are filled to capacity, expression as percentage of fresh or dry material can indicate only little starch. With respect to nitrogen nutrition, such a plant is not considered deficient in carbohydrates, even though the absolute amount may be low.

On the other hand, macroanalyses of a black-green succulent plant may indicate much more starch than in the yellow-green woody one, on an absolute-amount basis, or as percentage of fresh or dry material. Microscopic observation, however, shows that such plants are provided with only about 25 per cent of the starch their tissues are capable of holding. Such a plant is considered defi-

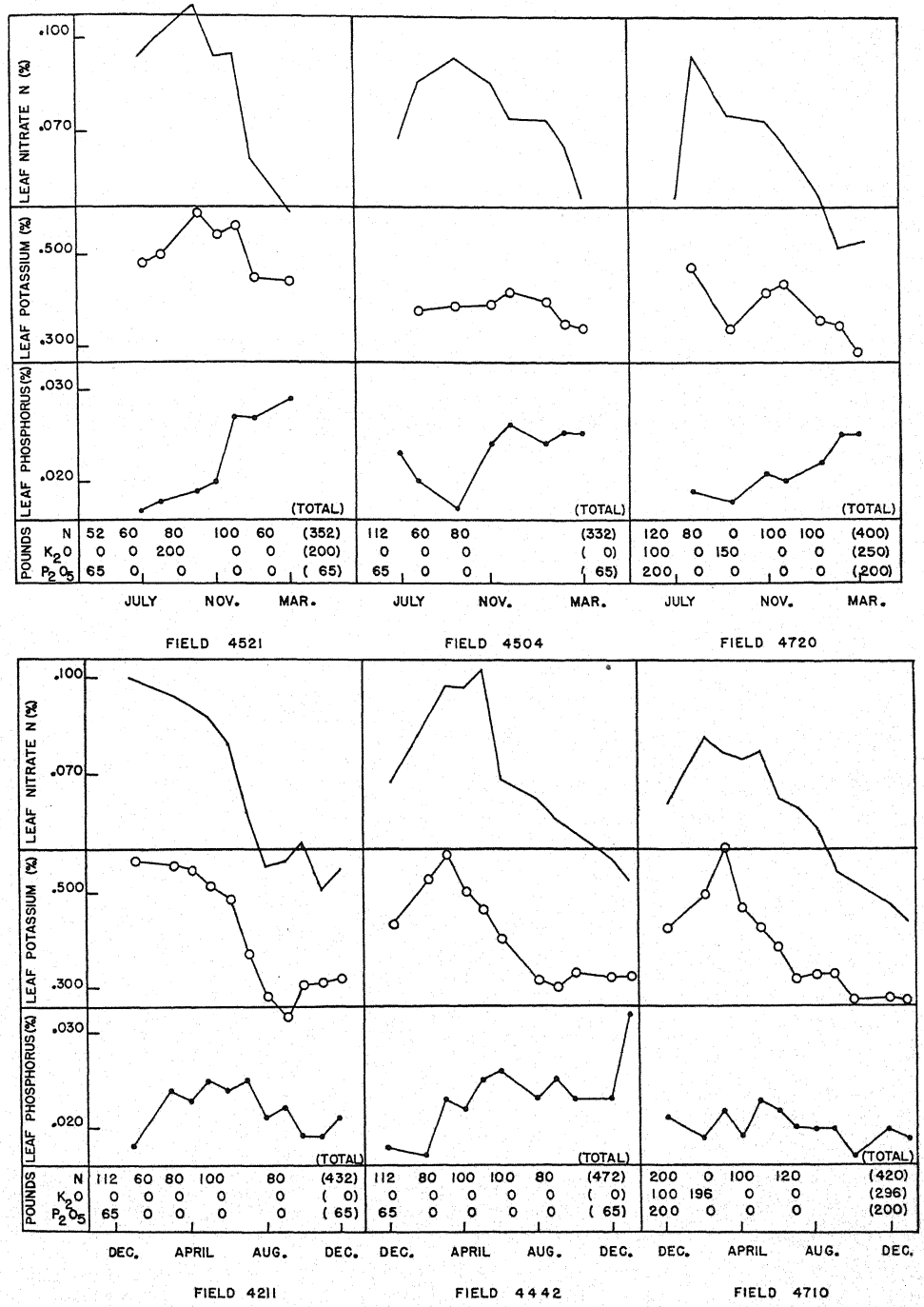
cient in carbohydrates, even though the absolute amount of starch may be higher than in the former case. Microchemical observations have shown that the degree of greenness of the leaves reflects to what percentage of capacity the plant is supplied with starch reserves. The yellower-green the plant, provided it is not senescent, the higher the starch content in the sense noted. The color standards employed for this purpose, significant within a range of plus or minus 3 per cent, are given in an earlier report (37).

Figure 1 shows the proximate maximum leaf-nitrate values attainable, but maximum nitrate reserves are desirable only if plants contain an adequate carbohydrate supply. This is indicated by about 15 per cent no. 1 yellow-green leaf color. To be conservative in the use of nitrogenous fertilizer in a location where past sunshine records indicate a low light rate, a greater carbohydrate reserve should be permitted to accumulate (about 30 per cent no. 1 color) before additional nitrogen is applied.

To prevent serious losses in yield of fruit it is particularly important to avoid either nitrate or carbohydrate deficiency at time of differentiation. In contrast, during earlier months of vegetative growth, plants may become so low in nitrate that they display 30–40 per cent no. 1 color with only relatively unimportant losses in yield, provided nitrate deficiency is rather promptly corrected. In interpretation of figures 4–24 these standards should be kept in mind, for the recorded percentages of leaf-nitrate nitrogen and no. 1 color make apparent either nitrate or carbohydrate deficiency where they appear as limiting factors in the experiments that follow. The percentages of leaf nitrate taken alone mean little, but extremely small differences in nitrate content become highly significant as associated with fruitfulness when considered in relation to carbohydrates (37). CHAPMAN and LIEBEG (12), in studies of the nutrition of citrus trees, recently obtained apparently similar results. They found that nitrogen content was not a reliable index of nitrogen requirements. They report "fluctuations in the dry weight yield of plants or plant parts, of nearly identical nitrogen content."

Discussion of results

Figures 2 and 3 show the characteristic relationships in nitrate, potassium, and phosphorus reserves which during a period of several years have appeared repeatedly in all similar routine records of fields over wide ranges of climatic and soil conditions. Analyses of the plants commenced soon after the root systems became established and there was noticeable growth of leaves. Analyses were discontinued at the time of differentiation of flower buds or shortly thereafter. The nitrate levels in the plants were much higher in some fields than in others. As already explained (37), the percentage of nitrate needed for maximum production varies with opportunity for carbohydrate accumulation. In none of these fields was nitrate a factor materially limiting production of fruit at the levels of available potassium and phosphorus. Carbohydrate synthesis, of course, depends on sunshine, temperature, and other factors which varied greatly among the areas in question. Although records of sunshine, temperature, rainfall, and soil composition are available, they are in general omitted from this discussion. They are peculiar to the site in question and often characteristic of it only for the season concerned. In contrast, quantitative plant records furnish an integration of the most complex environmental pattern



FIGS. 2, 3.—Leaf analysis in fields indicated, first taken about 3 months after planting and continued until early stages of blossom-bud development, showing intimate relationship between nitrate and potassium curves, except in field 4504 where no potash was applied and soil was very low in this element. Note typical inverse relationship between nitrate and phosphorus curves, except in fields 4211 and 4710 where available external phosphorus supply was extremely limited.

and supply information employable under a wide variety of conditions.

The absorption of anions such as nitrate is frequently greatly accelerated by the presence of rapidly absorbable cations such as potassium (1, 2, 8, 13, 19, 26, 32, 33, 51), and under field conditions nitrate is not absorbed freely when the potassium supply is low. Absorption of the ammonium ion is not favored by potassium (2, 15, 35, 36). But, regardless of the form of the nitrogenous fertilizer applied, under field conditions nitrate is almost the sole external source of nitrogen. Exceptions would be found where soil has recently been wholly or partially sterilized and in flooded areas, as in the case of rice paddies. In both cases oxidation of reduced forms of nitrogen to nitrate might be limited (36). As an agricultural procedure, however, potash is now applied to pineapple fields with (in part) the specific objective of increasing the nitrate reserves in the plant. There must be an external supply of nitrate, of course, but heavy additions of nitrogen to the soil are often associated with low nitrate reserves in the plant unless coupled with an adequate supply of potassium. The typical, closely corresponding curves for leaf nitrate and leaf potassium are shown in figures 2 and 3. There is not perfect correlation in all cases between leaf values for nitrate and potassium, nor would it be expected. Other cations undoubtedly play an important role. This would be true perhaps of such a field as 4504 (fig. 2), which was extremely low in available soil potassium and received no potash applications. Under weather conditions favoring carbohydrate accumulation, high leaf-nitrate values are required (37), as in the earlier stages of growth of field 4521 (fig. 2). But in order that the needed nitrate may be absorbed, a relatively great supply of potassium is essential. Potassium has other functions than merely accelerating the absorption of nitrate, and other cations often favor the intake of anions (18, 22, 23, 44, 46); but under a great variety of soil conditions there seems no practical substitute for potassium in its relationship to absorption of nitrate by the pineapple plant. On the other hand, where carbohydrate content is limited because of weather conditions, and in consequence only a low nitrate reserve is required, a leaf-potassium percentage which does not fall much below 0.380 seems adequate for other functions of potassium.

These relationships will not necessarily be directly displayed by all species of plants under all circumstances, as pointed out by HOAGLAND (22) and emphasized by BECKENBACH and others (4). For instance, under conditions of extreme potassium deficiency, plants of a type that store nitrate may ultimately become filled to capacity with it, particularly where sodium is substituted for potassium. This is probably not because of high rate of absorption of nitrate, for the plants increase in volume very slowly, but because of limited use of nitrate in new protein synthesis (24, 38). The potassium requirements of species that store nitrate in quantity may well be different from those of plants rarely containing more than traces (36). The only cation considered in these experiments is potassium, yet experiments with solution cultures and field tests have shown that high levels of potassium nutrition may be associated with calcium deficiency. Conversely, heavy applications of calcium often result in lack of sufficient potassium in the plant (6, 14, 16, 17, 22, 29, 33, 41, 54).

Nevertheless, under field conditions the pineapple plant apparently demon-

strates the allied absorption of nitrate and potassium. The commonly found inverse relationship between intake of two ions of like sign is indicated in figure 2 by the curves for leaf phosphorus and nitrate. This relationship will not always find expression, if there is a severely limited supply of available phosphate in the soil, as in some of the fields shown in figure 3. Many workers have found that, by decreasing the concentration of nitrate, plants may often be induced to accumulate more phosphate. Conversely, a high level of phosphate nutrition is sometimes associated with nitrate deficiency (1, 2, 10, 12). Under field conditions in the case of most crops, however, relatively heavy applications of phosphate are required before absorption of nitrate is noticeably depressed. On the other hand, any superphosphate or nitrogen applied to pineapple fields after planting is commonly not distributed in the soil around the plant but is placed in a localized zone—in the axils of one or two of the lower leaves (37). This may lead to a greater suppressing action of phosphate on absorption of nitrate than might otherwise occur, although the limiting effect of nitrate on intake of phosphate is more commonly in evidence. Apparently when climatic conditions favor carbohydrate accumulation and a high nitrate reserve is accordingly required (37), absorption of phosphate may be suppressed. In consequence, more liberal applications of phosphate might be needed than if the light rate were lower (and the needed nitrate therefore less), as in another season in the same site.

During earlier vegetative stages a leaf-phosphorus content of 0.020 per cent or somewhat less may be adequate. With diminution in nitrate reserves just prior to and during blossum-bud differentiation, phosphorus tends to increase in the great majority of cases, unless the available supply in the soil is extremely low. At that time, for greatest possible yield leaf phosphorus should be about 0.028 per cent, provided there are no other limiting factors, such as nitrate deficiency. When other deficiencies are corrected severe losses in fruit production occur when phosphorus reserves are below this level at differentiation. Until recently, phosphate applications just prior to differentiation were not considered feasible commercial procedure, for repeated experiments gave contradictory results. These earlier experiments in conjunction with soil analyses, however, did not include any records of the plants except yields. Fruit weights from each of six replicates were taken separately. But statistical analysis showed in some seasons highly significant gains and in others significant losses from phosphate applications just prior to differentiation. The data that follow appear to furnish an explanation. For example, in the case of field 4521 (fig. 2) leaf phosphorus at differentiation in March was at the desired value of 0.028 per cent. If a late winter application of phosphate were made to this field, it might suppress nitrate absorption, resulting in deficiency of this nutrient in relation to carbohydrates. In consequence there would probably be a decrease in production of fruit (37). Field 4211 (fig. 3) presents a contrasting situation. There phosphorus reserves were very low at differentiation, and added phosphate would doubtless have increased yields greatly, provided the external supply of nitrate and potassium was maintained at a level high enough to prevent their serious deficiency.

Unquestionably other anions also play a significant part and may be limiting factors in the culture of the pineapple

plant. In fields such as 4710, for instance (fig. 3), where there is comparatively heavy rainfall, considerable quantities of sodium chloride may pass through the soil. In some fields as much as 500 pounds per acre per year, owing to ocean spray, are transported by the wind and carried to the soil by rain. Chloride and other anions have been reported to suppress absorption of phosphate and nitrate (12, 23, 30, 33, 60), and chloride may well have been a factor in field 4710, together with sodium, which would not be expected to favor intake of potassium. As information is lacking on many essential as well as nonessential ions absorbed by the plants in these experiments, complete interpretation of results is not attempted. Also, intact plants can never furnish measurements of absorption that are distinct from metabolic activities and growth. The results of more precise studies of absorption, conducted by STEWARD (49) and others (18, 44, 46), appear in many cases to supply logical explanations of some of the responses here cited.

The quantitative chemical and physical records (37) to be discussed were taken on plants in plots that received various fertilizer treatments with ammonium sulphate, potassium sulphate, and superphosphate (figs. 4–24). The experiments were of the usual agronomic type, with six replications of each treatment. These plant records are informative and are presented in part in the following pages. These results have since made possible the installation of field experiments wherein fertilizer applications were not arbitrarily predetermined but were made on the basis of plant composition, past weather records for the site concerned, and absorptive capacity of prevailing root systems. The objective in these newer tests is to learn how to attain efficiently, at different stages of growth, desired concentrations of carbohydrates, nitrate, potassium, and phosphorus, and their relationship to production of fruit. The growth responses of plants in these later experiments have furnished striking evidence that the previous conclusions are essentially correct. However, fruit from these later tests will not be ready for harvesting for some months. Whether or not fruit weights may then be obtained under present conditions is uncertain.

EXPERIMENT 55-C; FIELD 4705.—This field was planted in April, 1939. Records of the plants were first taken about 6 months later and were continued until March, 1940, when blossom-bud differentiation occurred. All treatments (figs. 4–9) except the K plots had a fairly liberal reserve of nitrate in relation to carbohydrates. This is shown by the percentage of no. 1 leaf color, which at no time was much greater than 15 per cent, the arbitrary point below which carbohydrate deficiency is indicated (37). Thus in all plots except the K series (fig. 4) neither nitrate nor carbohydrate deficiency limited production of fruit; that is, higher levels of nitrogen nutrition would not have increased yields under the prevailing climatic conditions and at the levels of potassium and phosphorus nutrition imposed.

Correlated with relatively low leaf potassium, the A plots were consistently lower in content of nitrate than the D (fig. 5). This is not unexpected, for potassium and nitrate absorption are commonly found intimately allied in many kinds of plants (1, 2, 8, 15, 22, 26, 28, 32, 33, 36). The relatively low level of nitrate in A, however, was not associated with greater carbohydrate accumulation than in D, as would be anticipated if carbohydrate synthesis had occurred at an equal rate in both series. Rather, no. 1

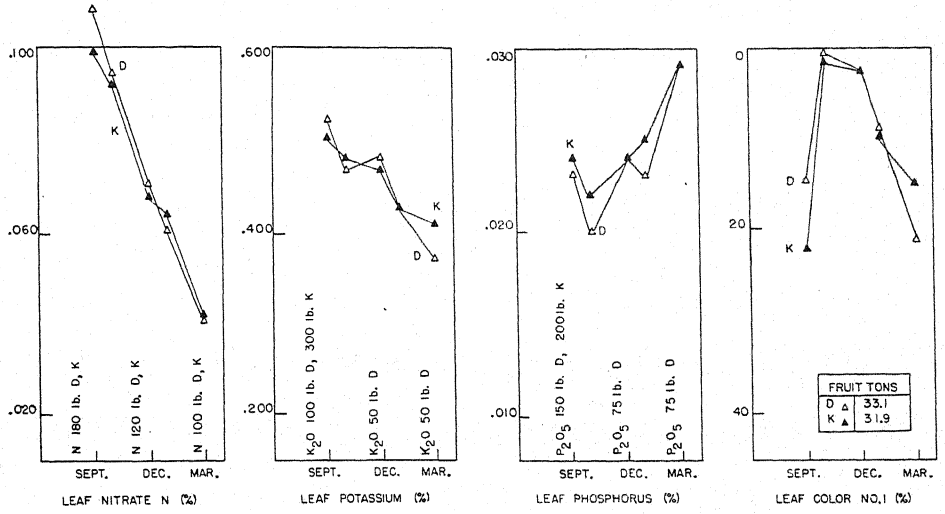


FIG. 4.—Experiment 55C, field 4705. Associated with extreme nitrate deficiency during first 6 months of growth, prior to obtaining formal records in September, are lower yields of K plots. In September, even after 6 months, note much lower nitrate and higher no. 1 color (higher carbohydrates) in K than in D plants. Leaf potassium and leaf phosphorus adequate in both groups. During early months high total concentration of fertilizers and high phosphate probably limited nitrate absorption by K plants (**39**).

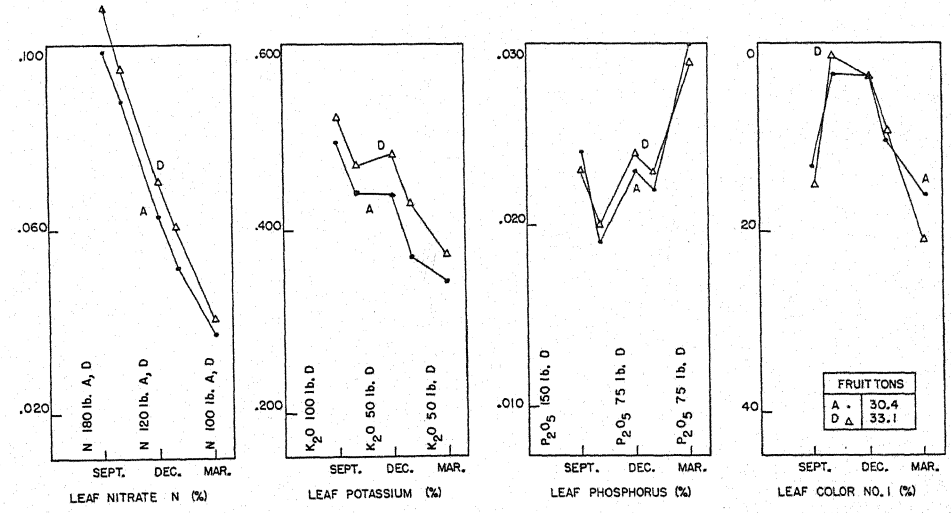


FIG. 5.—Experiment 55C, field 4705. Leaf phosphorus adequate in both groups and as high in A with no phosphorus fertilization as in D with added superphosphate. With lower leaf nitrate in A, note apparently freer absorption of phosphate (cf. fig. 6). Correlated with greater absorption of potassium in D than in A is consistently higher leaf nitrate. Nitrate proximately adequate in relation to carbohydrates, as in both A and D plots; no. 1 color not materially over 15 per cent, below which carbohydrates limit fruit production, not nitrate. Higher leaf potassium and leaf nitrate in D than A but no lower carbohydrate reserve (no. 1 color); hence larger plants and greater yields in D.

leaf color (indicative of carbohydrate content) was about the same in both until differentiation, when it was a little higher in D (fig. 5). That the lower concentration of nitrate in A was adequate in relation to carbohydrates and did not result in accumulation of a carbohydrate reserve large in relation to available nitrate may well have been due to the fact that the plants were limited in CO_2 assimilation by their relatively low content of potassium, an element which has repeatedly been found to be—directly or indirectly—essential for carbohydrate synthesis (3, 18, 24, 27, 38, 40, 42, 44, 46, 47, 48, 49, 56). In contrast, the D groups—with greater potassium supply and on that account a presumably more rapid photosynthetic rate—were apparently able to accumulate carbohydrates in approximately the same concentration as in A. This occurred even though growth of the D plants was greater and their nitrate reserves materially higher than in A. It would seem to follow that the use of carbohydrates in new protein synthesis from nitrate, known to be facilitated by potassium (8, 18, 24, 32, 38, 42, 44, 46, 47, 48, 49), while obviously greater in D than in A (as shown in part by greater growth), was compensated for by more efficient photosynthesis in the former case. The net results were much larger plants and nearly 3 tons more fruit per acre in the D than in the A plots. This was true, even though the concentration of carbohydrates and probably organic nitrogen was much the same in both groups of plants and the quality (not the size) of their fruit very similar, at least with respect to such factors as flesh texture and color and percentage content of sugars and total acids.

Thus, although the leaf-nitrate values were not the same in all plots, they were adequate in relation to carbohydrates (except in the K group) during the early stages of growth. In this treatment very heavy applications of potassium and phosphorus during the early months of the experiment, prior to obtaining formal records, were associated with extremely yellow leaf color. This may have been due in part to typical limiting effects of a high concentration of salts on absorption and assimilation of nitrate (39), brought on by the application of relatively large amounts of fertilizer. Also, the comparatively heavy application of phosphate to the K plants may have contributed to suppression in intake of needed nitrate (1, 2, 10, 11, 12, 13, 15, 23, 30, 33, 36, 42, 50, 51, 57, 60). In any event, the net result was extreme nitrate deficiency during the first half year of growth, which was not fully corrected at 6 months after planting. Even at that time, when the first analyses were made in September, the plants of the K plots were far lower in nitrate than those of the D and much higher in carbohydrates, as shown by a materially greater percentage of no. 1 color (fig. 4). The K series were equal to or higher than the D in potassium and phosphorus content but were outyielded by the D, undoubtedly because of nitrate deficiency in relation to carbohydrates, which was far more severe during the early months than is indicated by the first plant records taken in September.

In experiment 55-C, field 4705, for odds of 19:1 there is required a difference in yield between any two treatments of 0.83 tons of fruit per acre. As shown in figure 6, the B plots outyielded the A by 1.5 tons. The lower nitrate content of the A plants was not a limiting factor, for their carbohydrate reserves were too low to warrant a higher concentration of nitrate, as shown by the fact that at all times no. 1 color was 15 per cent or lower (37). Neither was phosphorus deficient

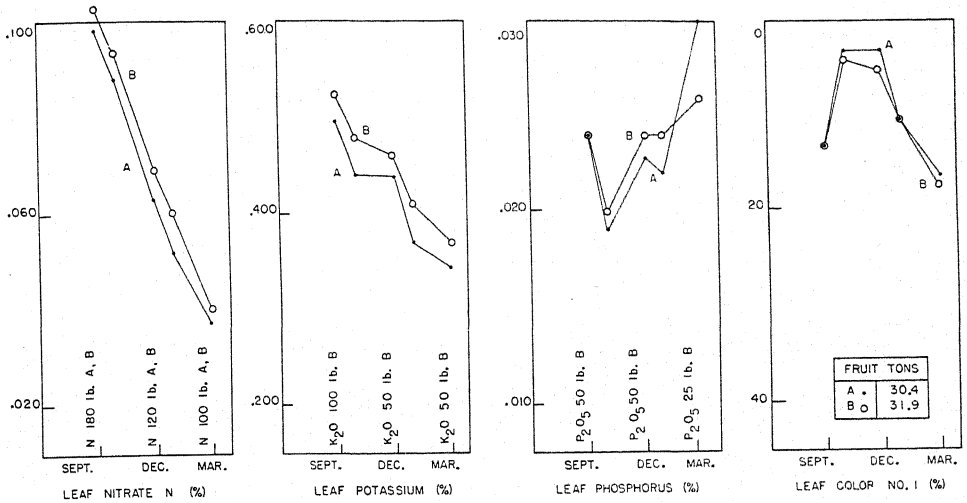


FIG. 6.—Experiment 55C, field 4705. At differentiation in March, higher leaf phosphorus in A than B, though only latter fertilized with phosphorus. Lower nitrate in A associated with freer absorption of phosphate. With same nitrogen fertilization, greater potassium absorption by B, correlated with consistently greater intake of nitrate; but B no lower than A in carbohydrates (no. 1 color). Potassium deficiency limited yields in A because of less absorption of nitrate and apparently lower plane of photosynthetic activity than in B.

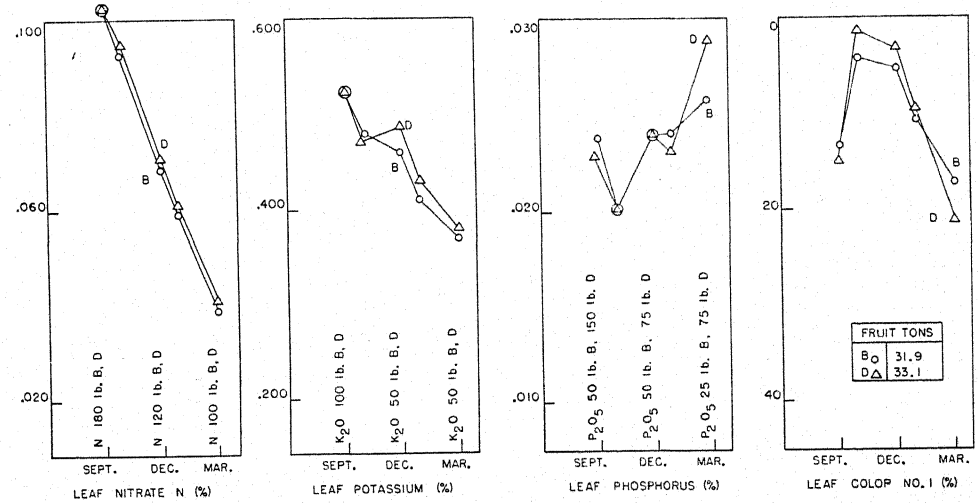


FIG. 7.—Experiment 55C, field 4705. D plots outyielded B because at critical period of differentiation in March D was at desired level of 0.028 per cent leaf phosphorus, with B definitely deficient. Essentially no difference in nitrate concentration between treatments but slightly higher potassium content of D may have contributed to significant but only slightly higher carbohydrate reserves (greater no. 1 color) in March. This might advantageously have been coupled with slightly higher leaf nitrate.

in A, even though no phosphate was added to the plants of this series. In fact, coupled with lower nitrate than in B, and presumably in consequence less suppressing action on phosphate intake, it was absorbed more freely by the A plants during the critical period of differentiation in March than by the plants of the B plots. Probably there occurred for this reason a leaf-phosphorus value of 0.030 per cent, much higher than in B, even though only the latter was fertilized with superphosphate. Clearly the B plots were more productive than the A, owing to their higher content of potassium, as shown by the leaf analyses for this element (fig. 6). Coupled with higher potassium in the B plants was a higher reserve of nitrate than in A. This invariably seems to occur in pineapple plants under field conditions, when there is available an adequate external supply of nitrate in the soil. But although the concentration of nitrate in the B plants was relatively high, their carbohydrate content, as shown by percentage of no. 1 color, was no lower than in the A plots—a situation already discussed in connection with figure 5.

But the B plots were in turn outyielded by the D group that produced 1.2 tons more fruit per acre. Reserves of nitrate, potassium, and carbohydrates, the latter indicated by no. 1 color, were much the same in both series (fig. 7), the most important difference being in the leaf-phosphorus content of the D plots at differentiation in March (0.029 per cent as compared with 0.026 for B). That the leaf-phosphorus value of 0.029 per cent was adequate is shown by the fact that a higher phosphorus reserve in E at differentiation (fig. 8) gave no increase in yield over D, with other determined factors essentially the same. Likewise the H plots (fig. 9), which varied from the D

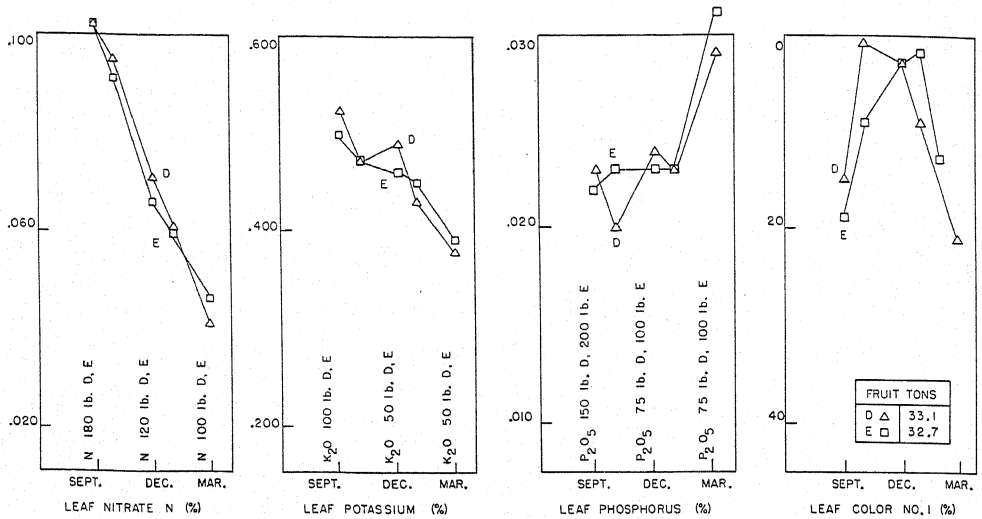


FIG. 8.—Experiment 55C, field 4705. No significant difference in yields. Leaf potassium adequate as about 0.380 per cent, or higher, at all times and similar in both treatments. In each series nitrate proximately adequate, though a little low in E plants only in September and low in D only in March, in relation to carbohydrates (no. 1 color). Leaf phosphorus in E higher than 0.028 per cent at differentiation did not increase yields.

chiefly in having a higher leaf-potassium content, indicate that potassium levels in D were adequate, for the yields of the H plots were not significantly increased over the D by their higher reserve of potassium. But the D plots at differentiation, when leaf potassium was lowest, still contained about 0.380 per cent.

For maximum yields in field 4705, under the climatic conditions prevailing, it was essential to fertilize with nitrogen, potassium, and phosphorus. In another year in the same location, however, if conditions were less favorable for carbohydrate synthesis, a lower level of nitrate nutrition would be indicated. Under these conditions it is doubtful whether phosphorus fertilization would be needed, as with a lower nitrate supply phosphate would probably be absorbed more freely, as in the plants of the A plots (fig. 6). With respect to potassium, considering the experimental results just discussed and those that follow, if in another year—because of lower carbohydrate content—lower nitrate reserves were required, less potassium would be needed for adequate intake of nitrate. In order not to limit metabolic activities such as protein and carbohydrate synthesis, however, for which potassium is directly or indirectly essential (3, 8, 18, 24, 27, 32, 38, 40, 42, 44, 46, 47, 48, 49, 56), where root systems and soil moisture permit, leaf potassium should probably not at any time be allowed to fall much below 0.380 per cent (figs. 7, 9), even though with a lower leaf-potassium content absorption of nitrate might be adequate in relation to low carbohydrate reserve.

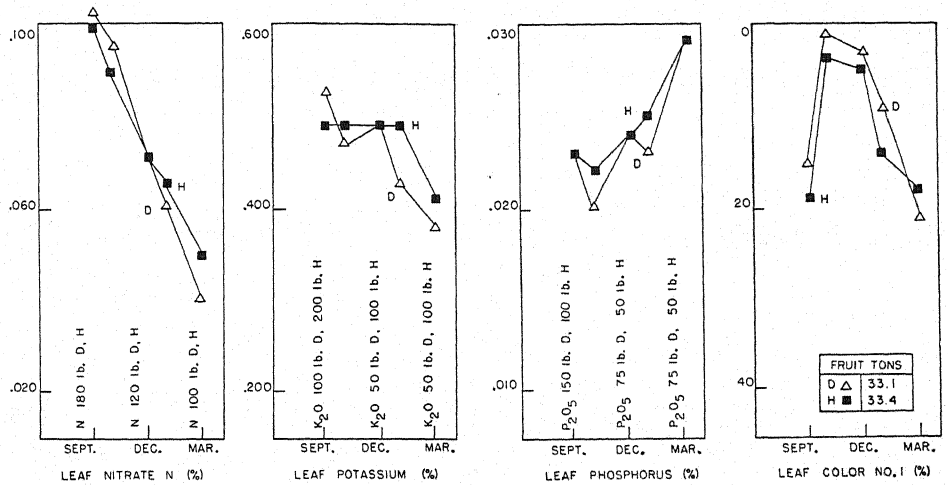


FIG. 9.—Experiment 55C, field 4705. No significant difference in yields. At differentiation in March leaf phosphorus at desired 0.028 per cent in both H and D. During vegetative period phosphorus 0.020 per cent or higher in both cases, hence not limiting factor. Nitrate in relation to carbohydrates (no. 1 leaf color) proximately adequate in both groups. Higher leaf potassium of H than D plots, December to March, did not significantly increase yields, indicating 0.380 per cent leaf potassium (as in D) adequate, where more is not needed for absorption of nitrate.

EXPERIMENT PK-3; FIELD 4703.—This field was planted in the fall of 1938, and the following spring records were taken of experimental plots until blossom-bud differentiation in December (figs. 10–13). While leaf-nitrate values were much the same in all plots and dur-

ing the spring approached the maximum concentration (fig. 1), they should have been somewhat greater, from March to June. This is indicated by the record for no. 1 leaf color, which in all plots was 40 per cent or more, indicative of high reserve of carbohydrates. However, the June application of 120 pounds of nitrogen per acre was associated with prompt and marked decrease in concentration of carbohydrate, as indicated by decrease in no. 1 color. In later months (and during differentiation in December), although leaf nitrate in all plots was much below capacity (fig. 1), it was nevertheless in every case very high in relation to carbohydrates, for the plants of all series displayed only about 10–15 per cent no. 1 color, indicative of a carbohydrate reserve on the borderline of deficiency.

The percentage of potassium in the leaves was about the same in all treatments and after June was extremely low, except in D and C in December. Potassium is apparently essential for free absorption of nitrate by the pineapple plant, as already pointed out. The low leaf-potassium values prevailing during the last half of the vegetative period were associated with a rate of absorption of nitrate which at that time was adequate in relation to carbohydrates, which were likewise extremely low. If there had been greater carbohydrate reserve, however, indicated by higher percentage of no. 1 color, and a higher nitrate content had in consequence been required, a more liberal potassium supply would probably have been essential for sufficiently rapid absorption of nitrate. Although the low potassium reserves of summer and fall, as in A and B, permitted accumulation of all the nitrate required, lack of potassium in later stages of growth was probably a factor adversely influencing new protein synthesis from nitrate and carbohydrate accumulation.

The comparatively yellow color of the

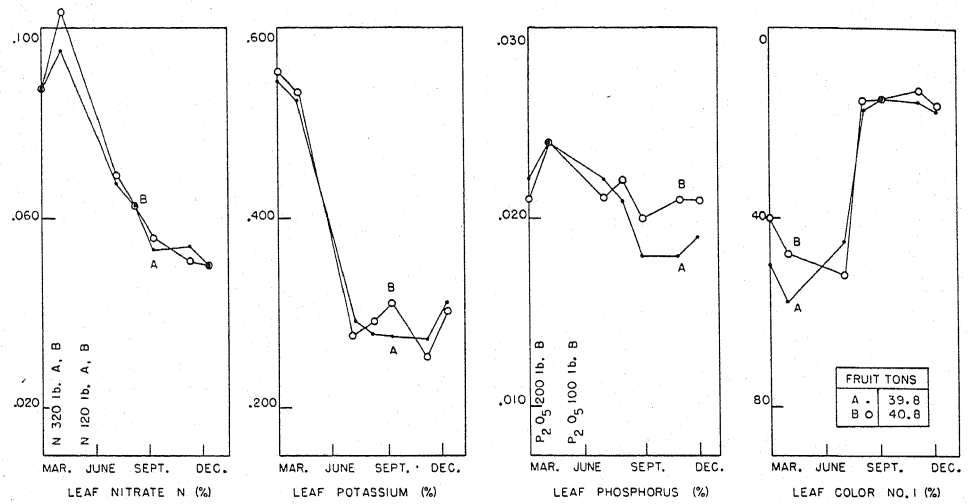


FIG. 10.—Experiment PK3, field 4703. After June, plants of both groups became extremely low in leaf potassium. During earlier months leaf nitrate too low, in relation to carbohydrates, no. 1 leaf color being about 40 per cent. Following June nitrogen applications, nitrate more than adequate, no. 1 leaf color remaining about 10 per cent. Greater yields of B correlated with inadequate but greater leaf-phosphorus values than A just prior to and during differentiation in December.

D plots from March to June (fig. 11) as compared with the others was undoubtedly a reflection of higher carbohydrate reserve, probably correlated with comparatively high soil-solute concentration, as the D plants in the earlier stages of growth received much more total fertilizer than any other group. A high salt concentration in the nutrient medium may induce carbohydrate accumulation (39). On the other hand, the greater supply of potassium and phosphorus available to D may have aided in synthesis of carbohydrates in the earlier months of growth, as already mentioned in connection with experiment 55-C in field 4705 (figs. 4–9).

With nitrate and carbohydrate reserves shown unimportant variables among treatments, except as indicated in the D plots, potassium and phosphorus may be considered in their relationship to yields, bearing in mind that there was required for odds of 19:1 a difference in yield between any two treatments of 0.94 tons of fruit per acre. As shown in figure 10, the B plots significantly outyielded those of the A. It is apparent that the potassium content in the two lots was almost the same and that the chief difference was in the greater leaf-phosphorus values of the higher-yielding B plants just prior to and during differentiation in December.

The D plots outyielded the B (fig. 11) but not enough for odds of 19:1. However, the slightly greater yield of D was correlated with slightly higher leaf-potassium values just prior to and during differentiation. In figure 12, the differences shown between treatments in leaf nitrate, leaf potassium, and leaf color (carbohydrates) were probably ineffective. This is indicated by the fact that when nitrate, only in September, was materially higher in D than in C, it was excessively high in both in relation to carbohydrates, as shown by the no. 1 leaf-color values. These were less than 15 per cent, indicating that both groups were

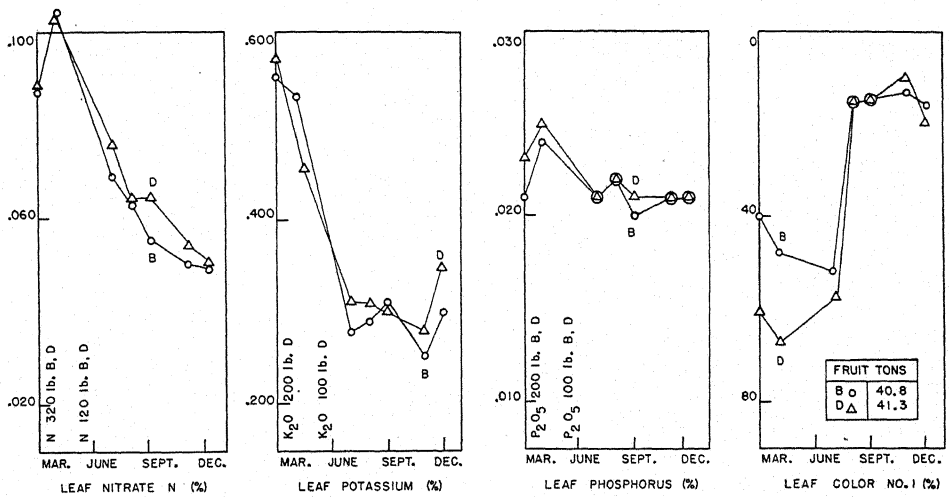


FIG. 11.—Experiment PK3, field 4703. Potash applications applied too early, for plants were then proximately filled to capacity with potassium (0.600 per cent leaf potassium). Slightly higher leaf potassium in D in winter months possibly beneficial, for D plots outyielded B, but not enough for odds of 19:1.

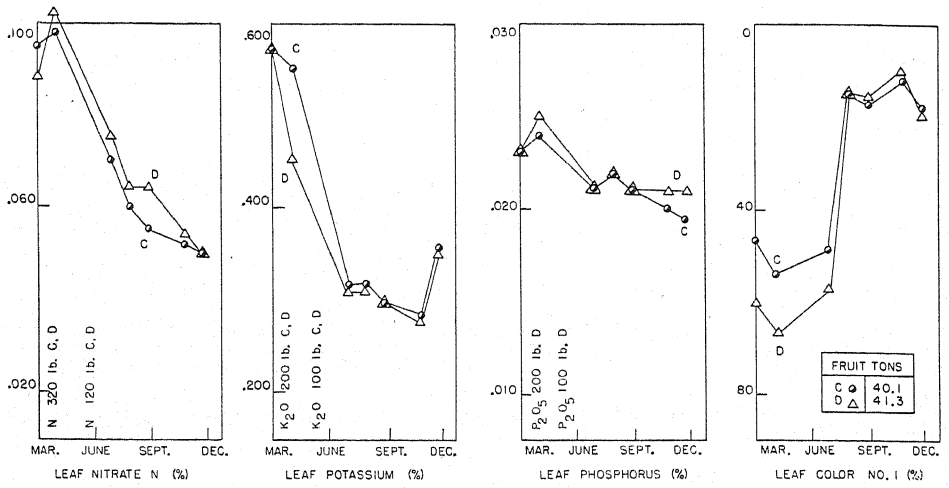


FIG. 12.—Experiment PK3, field 4703. Differences in treatments in leaf nitrate, leaf potassium, and leaf color (carbohydrates) probably largely negative in effect on yields, since when nitrate in September was significantly higher in D than C, nitrate was excessively high in both in relation to carbohydrates (no. 1 color less than 15 per cent in both). Leaf potassium higher in C only in April and then abundant in both series. D significantly outyielded C probably because of small but higher leaf phosphorus at critical period, just prior to and during differentiation in December (cf. figs. 7, 10, 13, 15).

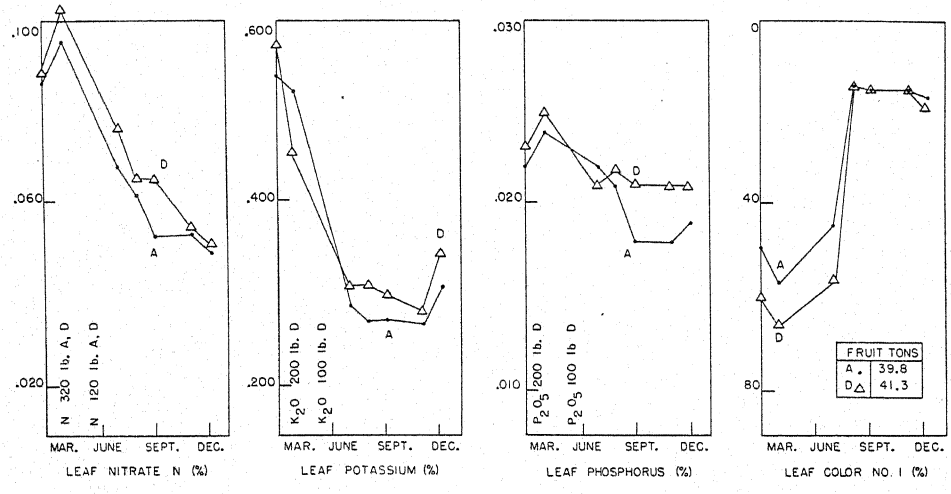


FIG. 13.—Experiment PK3, field 4703. Greater nitrate reserves in D than in A from March to June, when carbohydrates were high (no. 1 color over 40 per cent), undoubtedly contributed to increased yields. Leaf phosphorus far below desired 0.028 per cent at differentiation in December; but its much higher content in D than in A at that time probably major factor in higher tonnage of D.

on the borderline of carbohydrate deficiency, not nitrate deficiency (37). With respect to leaf potassium, it was materially higher in C than in D only in April, when there was an abundant reserve of this nutrient in both lots. The significantly higher yield of the D plots as compared with the C was probably correlated with the higher percentages of leaf phosphorus just prior to and during the critical period of blossom-bud development in December. It is recognized that the differences in phosphorus values referred to are small, but it has been demonstrated in many experiments, only a few of which can be presented in this paper, that very small deficiencies in phosphorus at differentiation have severe adverse influence on production of fruit (cf. figs. 7, 10, 13, 15). Comparison of the A and D plots in figure 13 further emphasizes the importance of adequate reserves of phosphorus at differentiation. As indicated in figures 4–9, 21, and 24, however, even the D series would probably have given much greater yields if leaf phosphorus had been up to 0.028 per cent at differentiation and leaf potassium had at no time been less than about 0.380 per cent (figs. 5, 18, 20, 22, 23, 24). During the earlier stages of growth, leaf potassium in the plants of the A and D plots was nearly up to capacity, about 0.600 per cent, even though no potash had been added. Also from March to August the A plots that received no phosphorus fertilization had approximately the same leaf-phosphorus content as the D. Effects of late applications of potassium and phosphorus would probably have been most informative. Apparently both potash and phosphate were applied at a time when neither was needed.

EXPERIMENT 55-C; FIELD 4217.—This field was planted in the fall of 1939, and records (figs. 14–17), first taken in January, 1940, were continued until blossom-bud differentiation in October. Figure 14 again emphasizes that under field conditions in Hawaii there is often indicated an intimate relationship between absorption of nitrate and potassium by the pineapple plant. Coupled with potash applications to the B plots and an increase in concentration of potassium in the plants as compared with A, the B groups were materially higher in leaf nitrate. This was true even though both series received the same nitrogen treatments. Even with much higher nitrate reserves, the B plots were not lower in carbohydrates than the A, for the percentages of no. 1 color were almost the same in both. Unquestionably the greater nitrate content of B favored more rapid utilization of carbohydrates in new synthesis of protein from nitrate, indicated in part by the greater growth and yields that the plants made. But that this did not result in lower carbohydrate reserves was perhaps correlated with favorable influences on CO_2 assimilation of the comparatively high potassium and phosphorus content of the B plots, already mentioned in the discussion of experiment 55-C in field 4705. Thus in neither A nor B was nitrate low in relation to carbohydrates, except in August, when the carbohydrate reserve was—for the stage of growth—high in both plots (about 30 per cent no. 1 color).

The phosphorus applications to the B plots materially increased leaf phosphorus over that of A, although at the critical period of differentiation in late fall both groups were extremely low in phosphorus, as compared—for instance—with the D plots in figure 7, which were up to 0.029 per cent leaf phosphorus at differentiation. The potassium and phosphorus values, materially higher in B than in A, were correlated with increase

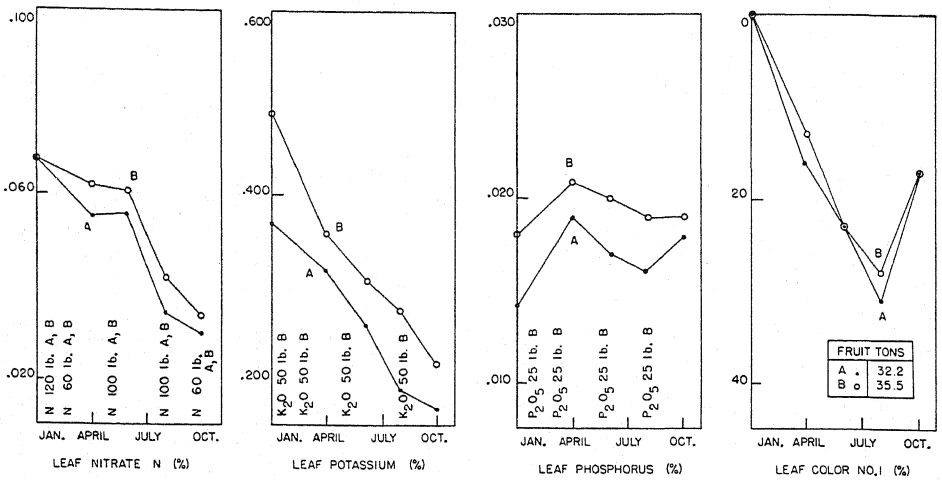


FIG. 14.—Experiment 55C, field 4217. Higher leaf potassium and greater intake of nitrate in B than in A, though both series had same nitrogen fertilization. Leaf nitrate in either series low in relation to carbohydrates (no. 1 leaf color) only in August and throughout concentration of carbohydrates about the same in both groups. Apparently higher potassium and phosphorus reserves favored greater CO_2 assimilation, as relatively high nitrate in B coupled with no lower carbohydrates, yet much larger plants and nearly 3 tons more fruit per acre, obviously required greater use of carbohydrates in new protein synthesis.

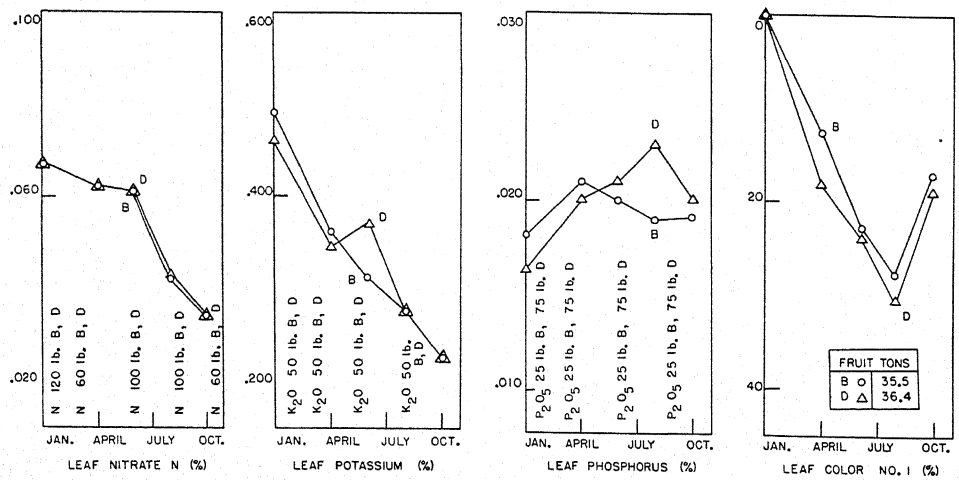


FIG. 15.—Experiment 55C, field 4217. That lower leaf phosphorus in B at differentiation in October limited production is suggested by higher phosphorus reserves in D at that time and greater yields, although differences in tonnage not great enough for odds of 19:1.

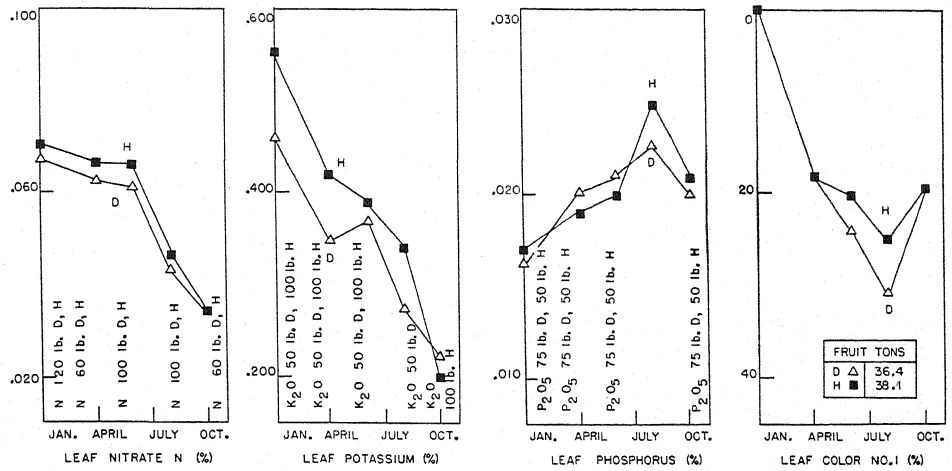


FIG. 16.—Experiment 55C, field 4217. Nitrate reserves adequate in both D and H until June, when higher potassium levels of H were coupled with greater nitrate absorption. In only D in midsummer was nitrate seriously inadequate in relation to carbohydrates, for then no. 1 leaf color was over 30 per cent. More favorable nitrate reserves in H, with more nearly adequate phosphorus at differentiation, contributed to higher yields than in D.

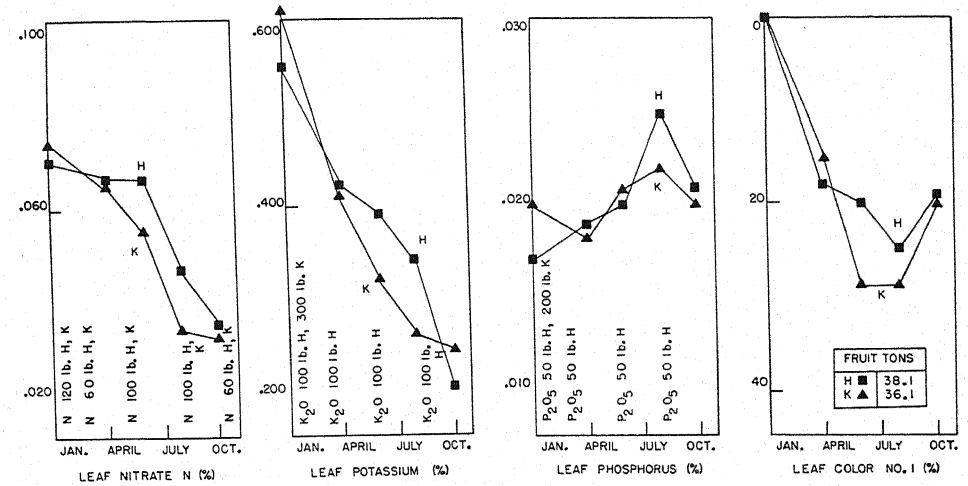


FIG. 17.—Experiment 55C, field 4217. From May to August K plots deficient in nitrate in relation to high no. 1 leaf color. Higher potassium reserves in H coupled with greater intake of nitrate than in K. Higher leaf phosphorus in H just prior to differentiation in October undoubtedly contributed also to 2 tons greater yield than in K.

in yield of more than 3 tons of fruit per acre, 1.2 tons being required for odds of 19:1 (fig. 14). That low leaf-phosphorus values in B at differentiation did apparently limit yield seems suggested by the D plots, which in their turn produced about 1 ton more fruit per acre than B (fig. 15). Although the D plots produced more fruit than the B group, the difference in production was not enough for odds of 19:1, owing to variance in yields within the six replicates. Nevertheless the most conspicuous difference in constituents was in the comparatively high leaf-phosphorus values of D as compared with B, just prior to differentiation.

The H plots (fig. 16) greatly outyielded both B and D and were highest in leaf phosphorus at the critical period, just before development of blossom buds. A major factor in the greater yields of the H plots was unquestionably their higher potassium content than in D or B. As usual, higher potassium apparently induces somewhat higher nitrate reserves (even though nitrogen fertilization was the same) and a more nearly ideal carbohydrate content, for no. 1 color during the summer in the H plots was not excessively high—as in the case of the D group. That the K plots were outyielded by the H (fig. 17) is probably due in part to deficiency of potassium in the former group, first apparent in June. This was correlated with inadequate absorption of nitrate in relation to carbohydrates, the K plots being much yellower (higher in no. 1 color) than the H, even though there was no difference in nitrogen fertilization. Evidently nitrate was not absorbed freely at that time, when the potassium supply in the K plants was limited (figs. 2, 3, 6, 14). Undoubtedly contributing also to the lower yields of K as compared with H were the lower leaf-phosphorus values prior to differentiation. The L plots (fig. 18), although much higher in potassium reserves than any other group and with no material lack in nitrate in relation to carbohydrates (except in September), were unquestionably limited by extreme phos-

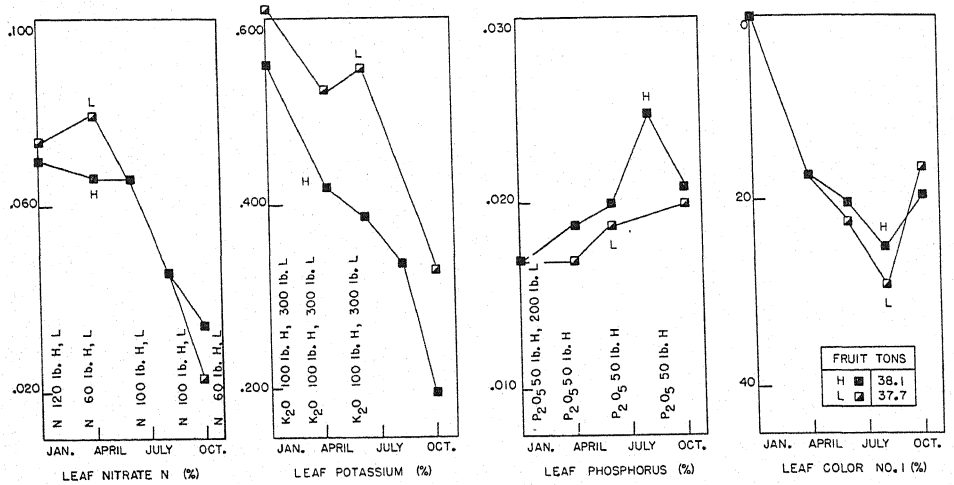


FIG. 18.—Experiment 55C, field 4217. Plants of L plots contained much higher potassium reserve than any other group but were probably limited in production, as compared with H, by relatively low leaf-phosphorus values, particularly just prior to differentiation in October.

phorus deficiency at differentiation, which probably counteracted the otherwise beneficial effects of the liberal potassium reserves. A desirable leaf-phosphorus content would have been 0.028 per cent at that time.

Maximum fruit production was probably not achieved in any treatment in experiment 55-C, field 4217, because in no plots were both potassium and phosphorus deficiency simultaneously corrected. This is based on the assumption that leaf potassium should at all times have been not less than 0.380 per cent and that leaf phosphorus at differentiation should have been about 0.028 per cent. The desirability of attaining these leaf values is indicated by the responses of plants in other experiments (figs. 4–9, 21, 24), as well as by the results of many other tests not here reported. Of course, if remedying these deficiencies had resulted in higher carbohydrate reserves, higher leaf-nitrate values would also have been required (37).

EXPERIMENT 55-B; FIELD 4710.—This field was planted in the fall of 1939. Analyses of plots were commenced the following spring and discontinued at blossom-bud differentiation in December. There was comparatively little difference in nitrate content of the plants of the several treatments (figs. 19–21). The B plots and the other potash-supplied series were slightly higher in nitrate than the A, undoubtedly owing to the usual favorable effect of the potassium ion on intake of nitrate by the plant. But in all plots throughout the experiment the nitrate reserves, although low as compared with the maximum values shown in figure 1, were nevertheless abundant in relation to carbohydrates. At no time in any case was no. 1 color materially over 15 per cent, and below this value approaching carbohydrate deficiency is indicated, not nitrate deficiency (37). The B plots (fig. 19) produced 1.5 tons more fruit per acre than the A, a difference just sufficient for odds of 19:1. Clearly this was correlated with the higher leaf-potassium and possibly leaf-phosphorus values in the B plots. At the critical period of differentiation, however, leaf phosphorus was too low, and there was but slight difference in the phosphorus content of A and B at that time. At earlier stages of growth, when leaf phosphorus was consistently higher in B than in A, it probably was largely without favorable influence upon yields, for 0.020 per cent leaf phosphorus (or possibly less) seems adequate during the period of vegetative expansion (figs. 4, 8, 15, 16, 17, 21, 24).

A comparison of plots B and H in figure 20 shows that the latter series significantly outyielded the B. The increased production of fruit was probably due mainly to the higher potassium reserves in the plants of the H plots. At differentiation in December, leaf phosphorus approached the desired value of 0.028 per cent in both B and H. Further indication that phosphorus was not directly a limiting factor, at least in earlier vegetative stages, is supplied by a comparison of D and H in figure 21, where yields were not significantly different. Here all determined constituents of the plants were practically the same in percentage values, excepting leaf phosphorus, which was materially higher in D than in H during vegetative development but not at differentiation in December. Then it was comparatively high in the plants of the H plots. This again indicates that during the period of vegetative growth only comparatively low leaf-phosphorus values are required—probably 0.020 per cent or somewhat less.

This experiment, while informative,

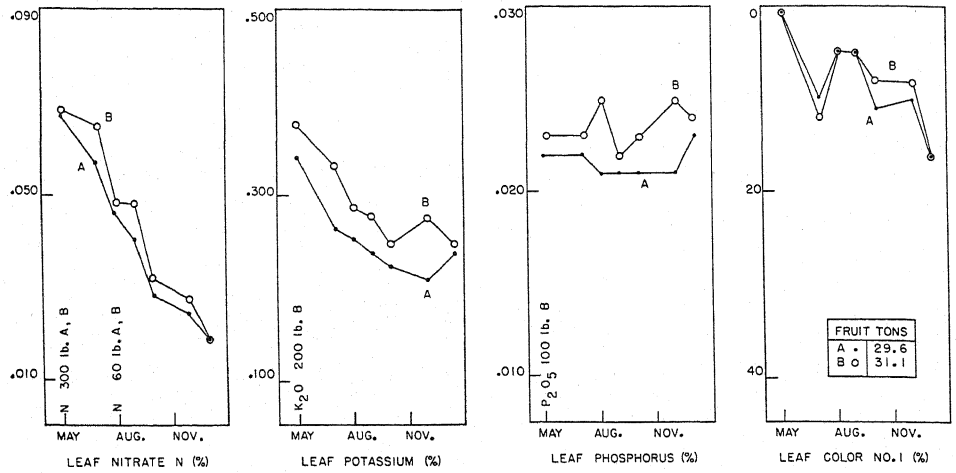


FIG. 19.—Experiment 55B, field 4710. With same nitrogen fertilization, higher potassium content of B plants was correlated with apparently slightly higher nitrate reserves, but carbohydrates were about the same in both A and B, and relatively low at all times (no. 1 leaf color 15 per cent or less). Clearly 1.5 tons more fruit per acre in B than A was associated with higher leaf potassium and possibly leaf phosphorus, though at critical period of differentiation in January A was nearly as high as B in phosphorus. Earlier higher phosphorus reserves in B were probably ineffective (cf. figs. 21, 24).

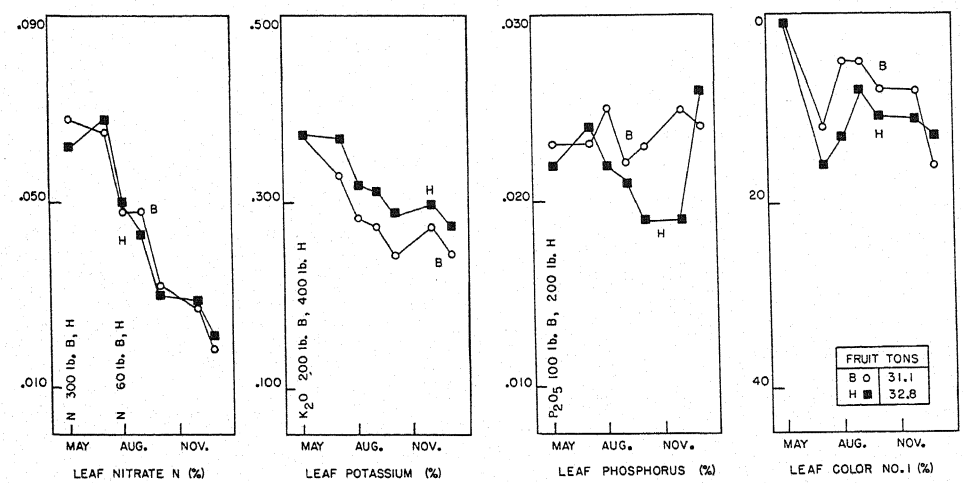


FIG. 20.—Experiment 55B, field 4710. Nitrate about the same and adequate in both B and H plants, as carbohydrates were low, not nitrate (no. 1 leaf color 15 per cent or less at all times). Higher potassium reserves in H brought increased yields over B and with higher potassium intake; carbohydrates, although low, were higher in H, July to December. At differentiation in January, leaf phosphorus, slightly higher in H, was probably advantageous.

seemingly fails to demonstrate potential possibilities in production of fruit for the season concerned in field 4710, chiefly because leaf-potassium values, while adequate for absorption of all required nitrate, should presumably have been not less than 0.380 per cent (figs. 4–9, 18, 20, 22, 23, 24). With more potassium available in later stages of growth there might have been accumulation of a carbohydrates than the B, indicated by a greater percentage of no. 1 color. It is highly probable that the potassium reserves, which were very low—even in the H plots—after August, materially limited synthesis of carbohydrates. Nevertheless, because absorption of nitrate was apparently also limited by lack of potassium, there resulted a condition of perhaps proximate balance between nitrate

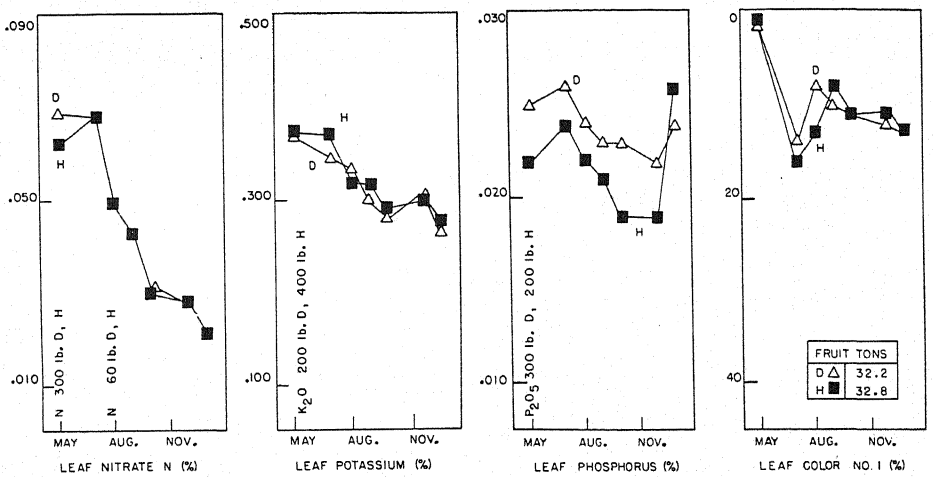


FIG. 21.—Experiment 55B, field 4710. With almost the same potassium reserves there was no essential difference in nitrate or in carbohydrates (no. 1 leaf color) between D and H plants. Relatively high leaf-phosphorus values in D only during vegetative growth (May to December) were ineffective, yields being not significantly different between treatments; but at differentiation, H was slightly higher in leaf phosphorus (cf. figs. 7, 10, 13, 15).

greater reserve of carbohydrates, which would have made desirable a greater supply of nitrate. This in turn might have made necessary more liberal applications of phosphate, in part because of the greater suppressing action that additional nitrate might have on phosphate absorption, already discussed.

Even the highest-yielding H plots (fig. 20) seemingly displayed a condition of proximately balanced multiple deficiencies (43, 51, 52, 53). For example, correlated with higher potassium content the plants of the H plots were higher in (and presumably organic nitrogen) and carbohydrates, with both on a comparatively low plane. On the other hand, the low level of nitrate nutrition doubtless favored absorption of the phosphate ion (fig. 2); even the A plants, unfertilized with phosphorus, absorbed this element rather freely, particularly at differentiation, when nitrate reserves were lowest (fig. 19). Commonly, pineapple plants with preconceived differential fertilizer treatments of the type indicated in the foregoing experiments produce fruit in any one site which, although varying

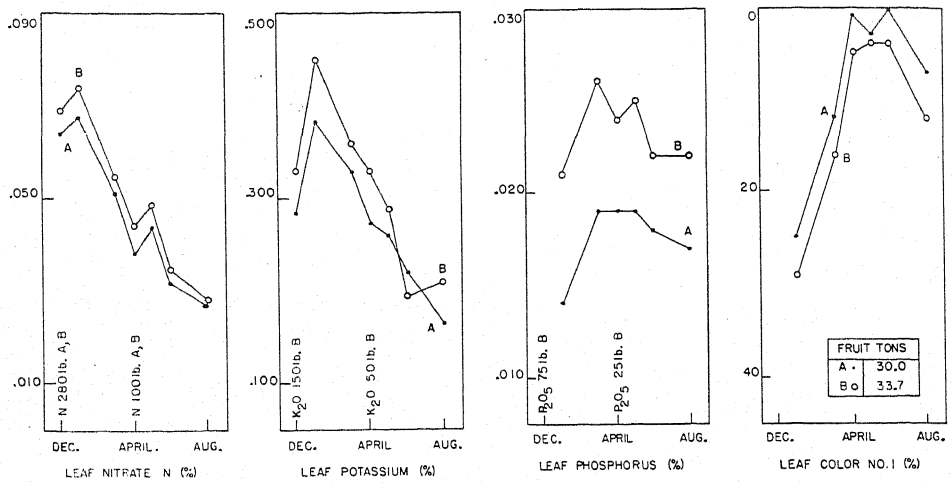


FIG. 22.—Experiment 55B, field 4720. Minor differences, though in right direction, are the higher potassium, nitrate, and carbohydrate reserves of B over A. Chief factor for 3.7 tons more fruit in B than A plots was higher phosphorus content. Leaf phosphorus unnecessarily high in B, from March to June, but should have been up to 0.028 per cent at differentiation in August (cf. figs. 7, 10, 13, 15, 24).

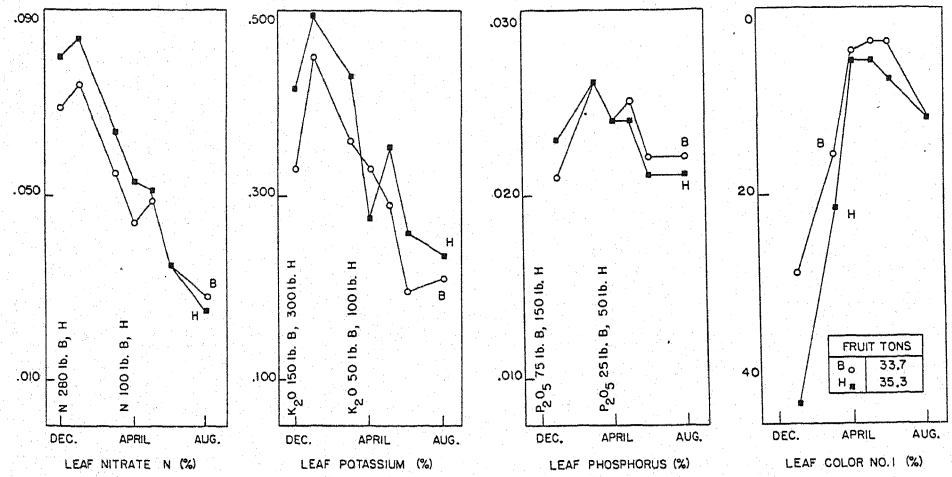


FIG. 23.—Experiment 55B, field 4720. That additional potassium as well as phosphorus was needed in field 4720 is indicated by increase in yield of 1.6 tons for H as compared with B. Except in April, leaf potassium was higher in H than B and induced higher nitrate early. High nitrate was then needed, for carbohydrate reserve (no. 1 leaf color) was relatively high in H. These factors apparently overbalanced favorable influences of slightly higher leaf phosphorus in B than in H at differentiation in August.

greatly in size among treatments, is—if harvested on the same date—often of about the same quality with respect to such factors as flesh color and texture, sugars, and acidity.

The interplay of compensating relationships in absorption and metabolism just mentioned would seem to suggest the reason for the little difference in fruit quality. These observations may be pertinent in relation to recent reports by MAYNARD (31) and HAMNER *et al.* (20) on the nutritional value of agricultural crops with various prescribed fertilizer treatments. For example, concerning ascorbic acid in tomato fruits, the latter workers conclude that "a macronutrient supply adequate to produce a fair yield is not a limiting factor in ascorbic-acid content." This seems to be in harmony with the responses of pineapple plants just described. Also they report that some of the tomato plants grown out-of-doors in sand culture produced fruits significantly higher in ascorbic-acid content than those grown in soil (20).

EXPERIMENT 55-B; FIELD 4720.—This field was planted in the early summer of 1938. Records were first taken in December and were continued through the period of blossom-bud differentiation in August (figs. 22, 23). Figure 22 shows that the chief factor for increase in fruit production of 3.7 tons per acre of B over A was the higher phosphorus content of the former group. Both the B and H plots had about the same leaf-phosphorus content (fig. 23); but that potassium as well as phosphorus was seriously deficient in field 4720 was evident from the significant increase in yield of 1.6 tons for H as compared with B, 1.5 tons being required for odds of 19:1. This gain was apparently correlated mainly with the leaf-potassium values in the H plots, which—except in April—were consistently much higher than in B (fig. 23). The higher potassium intake of the H plants for the same period was coupled with greater absorption of nitrate, until probably the external supply of nitrogen became very low in late summer, since no nitrogenous fertilizer was applied after March. Nevertheless, even in the H series nitrate reserves in relation to carbohydrates were very low prior to April. Carbohydrates during that time, as indicated by no. 1 color, were comparatively high in H, very likely owing in part to a favorable effect on carbohydrate synthesis of the relatively more liberal potassium reserves (figs. 5, 18, 20, 24). After June, with leaf potassium well below 0.300 per cent and a desirable concentration probably about 0.380 per cent, there was rapid decrease in carbohydrates, as indicated by the low percentage of no. 1 color in all treatments, so that if more nitrogen had been added to the plants in the later months it would have been largely ineffective even if absorbed (37).

Higher reserves of both potassium and phosphorus would evidently have been required during the summer months for maximum production. And if both potassium and phosphorus were applied and absorbed, with possibly an increase in carbohydrate synthesis resulting, heavier nitrogen fertilization might have been necessary to maintain adequate nitrate reserves and to obtain material benefit from additional fertilization with the other elements mentioned.

EXPERIMENT PK-3; FIELD 4817.—Records taken for a brief period were terminated at about the time of blossom-bud differentiation in April (fig. 24). Fertilizer applications gave negative results as far as yields were concerned, but the plant records are of considerable practical interest, in that in relation to produc-

tion of fruit they serve further to establish desirable levels of leaf potassium and of leaf phosphorus. While nitrate was not high as compared with the maximum values shown in figure 1, it was more than adequate; for carbohydrates, particularly at the critical period of differentiation, were low (only about 5 per cent no. 1 color in April). Accompanying greater intake of potassium by the plants proximately up to the desired level of 0.380 per cent.

There is some suggestion that the higher potassium—and perhaps phosphorus—content of the D plants was correlated with greater carbohydrate accumulation than in A, as shown by the somewhat higher percentage of no. 1 color of the D plants; but carbohydrate synthesis, if higher in D than in A, did not provide a

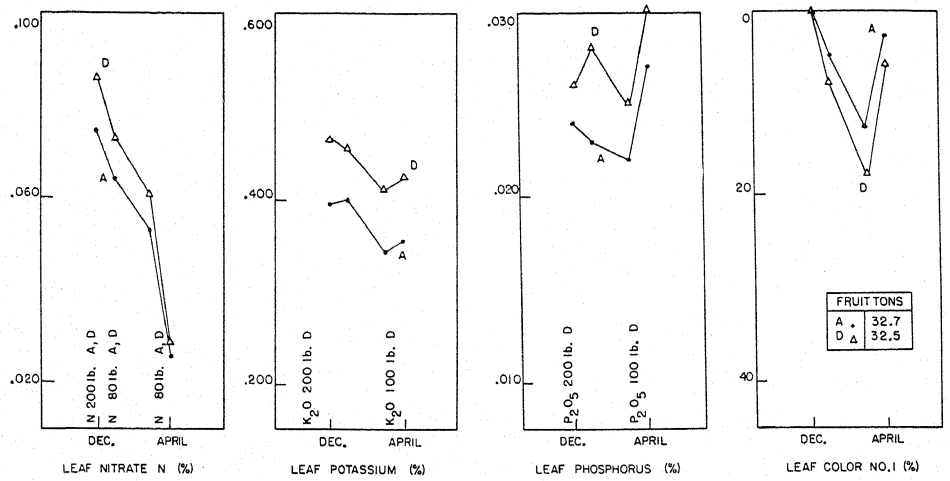


FIG. 24.—Experiment PK3, field 4817. Nitrate not a limiting factor in A or D, for carbohydrates were low (no. 1 color 15 per cent or less at all times). Much higher leaf potassium in D than in A gave no increase in yield; but leaf potassium in A not materially less than 0.380 per cent. With low carbohydrate content, potassium reserves of A were adequate for absorption of needed nitrate. At differentiation in April leaf phosphorus in A up to nearly 0.028 per cent and during vegetative period above 0.020 per cent, hence higher phosphorus content of D ineffective.

of the D plots, nitrate as usual was relatively high, but unnecessarily high in relation to the low carbohydrate content. Failure of the plants to accumulate a more liberal carbohydrate reserve would not be unexpected, however, as field 4817 is in a cloudy cool area with relatively heavy rainfall. Thus the higher nitrate reserve of D did not increase fruit production over A (37). The A series, which received no potassium fertilization, were also probably high enough in content of this element, for even at differentiation in early spring leaf potassium was apparently high enough reserve to warrant greater leaf-nitrate values than prevailed. As indicated in other experiments (figs. 5, 18, 20, 22, 23), it appears unnecessary to maintain a higher leaf-potassium content than 0.380 per cent, except where needed to facilitate absorption of nitrate, as when carbohydrates are high (37).

The phosphorus applications to the D plots during the months of vegetative development resulted in a striking increase in reserves of this element (fig. 24). At differentiation, however, even in the A plots (unfertilized with this element) leaf

phosphorus was 0.027 per cent—practically at the desired arbitrary value of 0.028 per cent (figs. 7, 8, 21). During earlier stages of growth the phosphorus content of A was much less than that of D, but at all times it was well above 0.020 per cent. There seems to be nothing to indicate that leaf-phosphorus values higher than 0.020 per cent are necessary during the vegetative period (figs. 4, 8, 15, 16, 17, 21, 24), provided leaf phosphorus reaches 0.028 per cent at differentiation. It may again be emphasized that in cases where both nitrate and phosphorus are below desired levels, correction of either alone might well bring about still further deficiency of the other, owing to possible competition for absorption between nitrate and phosphate (fig. 2).

Although the fertilizer treatments are clearly reflected in the leaf analyses, it seems obvious that neither potassium nor phosphorus was the limiting factor in field 4817. Under different seasonal conditions, however, even in the same location, if conditions were more favorable for carbohydrate synthesis higher levels of nitrate would be required, and probably a more abundant supply of potassium would be needed, for adequate absorption of nitrate. In turn, because of the additional nitrate, there would be expected a greater suppressing action on absorption of phosphate, with possible need for phosphorus fertilization.

Because of space limitation, additional figures illustrating other results are not given; those included represent but a fraction of the data which might be presented. While the plant responses have varied in pattern in different locations and in different years in essentially the same site, in no case have responses been obtained which have appeared to be aberrant in relation to those presented. However, situations have occurred that are not illustrated in the figures shown. While not of common occurrence, the following in particular would seem to deserve mention, even though further data will be required before drawing final conclusions. In experimental plots in certain fields in some years the addition of only nitrogen and potassium has given substantial increases in fruit production. In other plots in the same experiment, the application of only nitrogen and phosphate has given comparable gains in yield. But with the same nitrogen supply, treatment with both potassium and phosphate has in some cases given no greater increases in tonnage than either alone.

In cases where potassium has been the chief factor limiting yields, additions of it should increase fruit production. On the other hand, studies of interrelationships of nutrient ions (**18, 42, 44, 46, 49**) have demonstrated that phosphate (as well as nitrate) facilitates absorption of potassium. Phosphate fertilization, therefore, even though not directly needed, might induce greater potassium absorption, and sometimes does, as indicated by leaf analyses of pineapple plants not reported here. Of course the reverse situation might possibly occur, as potassium would be expected to contribute to greater absorption of phosphate and nitrate ions. With free intake of the latter, however, phosphate absorption would undoubtedly be severely repressed, at least in pineapple. There is considerable precedent for the foregoing (**13, 15**), and a report by LILLELAND and BROWN (**28**), working with peach trees, seems pertinent. They state in part, "potassium was increased in the leaf by applications of phosphorus. . . . . Growth increases in 1938 and 1939 could be correlated with potassium (in the leaves), although no potassium had ever been added."

There were only comparatively small

differences in time of blossom-bud differentiation and fruiting among the respective treatments in the experiments discussed here. Such experiments were chosen for presentation because, if any material difference had occurred in treatments in length of time available for vegetative growth, it would have been a



FIG. 25.—A plots (left): 370 lb. nitrogen, 120 lb. K_2O, and 185 lb. P_2O_5 applied per acre. C plots (right): 500 lb. nitrogen, 500 lb. K_2O, and 300 lb. P_2O_5 applied per acre. Essentially all plants in A plots display fruit, as compared with relatively few in C. Nitrate in relation to carbohydrates, prior to and during bud development, was low in A and relatively high in C plants.

dominant factor in determining the quantity of fruit produced. Pineapple plants with a relatively long period of vegetative expansion will almost invariably far outyield others in the same site with a shorter interval, except under extremely adverse nutritional or climatological conditions. Thus it would have been difficult, if not impossible, to have made direct comparisons of one fertilizer treatment with another in relation to plant composition and yields, if the respective plots within an experiment had varied greatly in time of fruiting.

As brought out earlier (37), during seasons when minimum temperatures are not low, plants deficient in nitrate and high in carbohydrates may fruit months earlier than others containing a more liberal reserve of nitrate but a lower concentration of carbohydrates. Nevertheless, plants apparently differentiate flower primordia simultaneously, regardless of material differences in nitrate or carbohydrates, provided minimum temperatures are sufficiently low (37). Figure 25, however, illustrates fertilizer plots wherein time of fruiting was in large part dominated, directly or indirectly, by available nutrient supply. These plots were planted on the same day, but the plants were widely different in time of blossom-bud differentiation and fruiting. The A plants, most of which fruited early, had at the initial stages of bud development 0.043 per cent leaf-nitrate nitrogen and a liberal reserve of carbohydrates (22 per cent no. 1 color). In contrast, the C plants, with relatively few displaying blossom buds, at that time contained 0.062 per cent leaf-nitrate nitrogen but only 11 per cent no. 1 leaf color. The latter symptom was in part an external expression of a relatively low concentration of carbohydrates (37). Comparable differences in nitrate and carbohydrate reserves between A and C were apparent weeks before the earliest stages of differentiation in the A plants.

The greater nitrate reserves of the late-fruiting C plots were associated not only with relatively liberal fertilization with nitrogen but with greater potash supply, as compared with A (fig. 25). Nitrate is not freely absorbed when potassium is low. This was apparent in bordering experiments, in the same field and planted

at the same time, where with low potash fertilization marked differences in nitrogen applications were without material influence on time of fruiting. The relatively heavy phosphorus applications to the C plots were apparently largely ineffective. At least at the time of plant analysis they were not reflected in higher phosphorus reserves than in A. This was presumably due to the repressing action on phosphate absorption of the relatively high nitrate content of the C plants (figs. 4–24), a situation repeatedly illustrated (1, 2, 10, 11, 12, 13, 15, 23, 30, 33, 36, 42, 50, 51, 57, 60).

Extensive chemical studies of pineapple plants varying in time of fruiting have not been many. In field experiments extremes of nutrition are not often employed, and where occasionally they have been, low temperatures in many cases have apparently resulted in practically simultaneous blossom-bud differentiation of plants of widely different chemical composition (37). Nevertheless, the interrelationships of carbohydrates, nitrate, potassium, and phosphate discussed in the preceding pages seem in harmony with the responses of other species of plants.

That length of night, or low temperature, or both may determine or greatly influence the time of flower-bud differentiation in many plants is well known. It is likewise recognized that under some environmental conditions an excessive supply of available nitrogen in relation to carbohydrates may often lead to limited fruit production, or to greatly delayed fruiting (25, 42). While the foregoing points have been emphasized frequently in the literature, the influences of potassium and phosphorus on nitrogenous and carbohydrate reserves and correlated plant responses have been less seldom considered. Such consideration should be given all elements considered essential.

For the reason already noted, heavier phosphorus applications to C than to A (fig. 25) were probably largely ineffective, as they were not reflected in concentrations of phosphorus in the C plants which were any higher than in the A group. But heavy applications of potassium and nitrogen may result in greatly delayed fruiting (fig. 25). This would be expected, for often when potassium is abundant there is relatively free absorption of nitrate, if a liberal supply is available in the soil. Furthermore, these results are in accord with observations by RUSSELL (42) that potassium and nitrogen are intimately linked in action. MCCALLA and WOODFORD also reported that limiting the potassium supply of wheat plants resulted in decreased nitrogen content (32, 33). HEPLER and KRAYBILL found that high potassium fertilization tended to delay blooming and ripening of tomatoes (21). These responses to high potassium nutrition, and many similar cases that might be cited, may be correlated with a comparatively high rate of nitrate absorption and reduction. If this is the case, there necessarily occurs relatively rapid oxidation of carbohydrates or their derivatives (35, 36), which under some environmental conditions might lead to deficiency of carbohydrate reserves and the characteristic plant responses noted.

Directly or indirectly, potassium unquestionably accelerates absorption and reduction of nitrate, with concomitant oxidation of nitrogen-free reserves (38). But potassium may induce an increase in carbohydrate (no. 1 color) in pineapple plants (figs. 4–24). Therefore the effect of potassium in bringing about more efficient photosynthesis is often to correct the adverse effects on fruit production of

excess nitrogen (42). Thus, particularly in a warm sunny area, potassium could well have the opposite effect on time of fruiting of that shown in figure 25.[1] As always, CO_2 nutrition, which varies with the weather, plays an important role in determining the responses of plants to soil fertilizer treatments, not only in the case of nitrogen (37) but of potassium as well.

That an adequate schedule for phosphate fertilization of pineapple fields cannot be accurately formulated in advance of knowledge of the nitrate requirements of the plants has been indicated (figs. 4–24). When little nitrate is employed, because carbohydrates are low owing to unfavorable weather conditions (37), phosphorus may be absorbed freely—even from soils low in this element (figs. 5, 6). On the other hand, in a year when carbohydrate reserves are high and nitrate is therefore required in abundance, much heavier applications of phosphate are necessary to counteract the suppressing action of nitrate on its intake. The responses of the plants presented in the illustrations of this paper are not aberrant in relation to those of other species. CHAPMAN (10) reports that "nitrogen deficiencies caused inorganic phosphate to accumulate within the oat plant, even in soils deficient in phosphate." MCCALLA and WOODFORD obtained comparable results in wheat (33). Frequently coupled with phosphate accumulation in high concentrations in plants low in nitrate is early senescence of leaves or of the plant as a whole (11, 58, 59).

[1] Although it would seldom be found under conditions of commercial productivity, extreme potassium deficiency, provided some functional leaves are present, sometimes leads to a brief period of carbohydrate accumulation and premature fruiting. This is apparently due, at least in part, to limited use of carbohydrates, in that new protein synthesis from nitrate is practically nil (38, 56).

HEPLER and KRAYBILL (21) found that heavy applications of phosphate hastened maturation of tomatoes, owing to early blooming. RUSSELL (42) emphasized that "phosphatic fertilizers hasten the ripening process." This ripening effect is said to be "well shown on the barley plots at Rothamsted; crops receiving phosphates are golden-yellow in colour while those on phosphate-starved plots are still green." WILLIAMS (58, 59), working with oats, reported that high phosphorus treatment resulted in early maturity, increase in dry matter, and reduction in protein nitrogen content, correlated—at least in part—with the retarding "effect of the high external concentration of phosphate on the intake of nitrogen by the roots."

These responses will not find expression under all environmental conditions (45, 55), as there are many possible limiting factors other than those considered which are often dominant (18, 42, 44, 46, 49). Nevertheless the responses are typical. In harmony with them, MCMURTREY (34) found that phosphorus deficiency delayed maturation of tobacco. Effects of phosphorus deficiency on the delayed ripening of oats and "the protracted and needless intake of nitrate" are reported by STREBEYKO (50). He also found that the higher the amount of phosphate supplied the earlier the plants ceased to take nitrate from the soil and the sooner the grain matured.

Under usual field conditions, it is much more common to find that nitrate limits the intake of phosphate than that the latter suppresses the absorption of nitrate. Even comparatively light applications of nitrogen, in years when weather conditions are not favorable for abundant carbohydrate synthesis, can bring about nitrate accumulation and repression of phosphate absorption in the pine-

apple plant. This is usually accompanied by a low level of carbohydrates and fairly vigorous vegetative growth. It probably is common in other plants. Possibly this situation is comparable with that described by MAYNARD (31). He reports areas where trouble with grazing animals is severe, but "both legumes and grasses grow luxuriantly and apparently provide liberal amounts of nutrient other than phosphorus."

Summary

1. In the pineapple plant a low reserve of nitrate was found adequate for greatest possible yields of fruit when carbohydrates were low. In contrast, when carbohydrates were high maximum production was not obtained unless the plants were essentially filled to capacity with nitrate. In practically the same location a difference of 75 per cent in nitrogen requirements was found in successive years (37).

2. When the concentration of carbohydrates is low and relatively little nitrate therefore supplied, phosphate is freely absorbed, even from soils rather low in phosphorus. In the same site under environmental conditions more favorable for carbohydrate accumulation more nitrate is needed. With higher nitrate there is a proximately corresponding suppression of phosphate absorption, so that it becomes necessary to apply phosphate.

3. The required level of nitrate nutrition, which varies with opportunity for carbohydrate accumulation, is intimately associated with potassium requirements. Under field conditions in Hawaii, nitrate is not absorbed freely by the plant when potassium is low. When carbohydrates are high, and relatively much nitrate therefore required, additional potassium is necessary for nitrate absorption, above that necessary for other functions that potassium performs. Even in the same site the amount of potash required varies with weather conditions from year to year.

4. In plants growing on soils notably low in potassium and phosphorus there was seemingly an interplay of compensating relationships. When potassium was low and absorption of nitrate was in consequence limited, carbohydrate accumulation was also curtailed. In turn, although no phosphate was applied, the low level of nitrate permitted fairly free absorption of phosphate. This apparently resulted in a condition of proximately balanced multiple deficiencies and greatly reduced size of plant and fruit. But, except for size, the plants were much the same in appearance as others with more liberal fertilization, and the fruits were about the same in physical characteristics and in percentage of sugars and total acid as those from plants with a more abundant nutrient supply.

5. Phosphorus is necessary in relatively high concentration just prior to and during blossom-bud differentiation, or severe losses in yields occur. An adequate supply of nitrate in relation to carbohydrates is also essential at that time (37). Application of phosphate to plants high in phosphorus and deficient in nitrate might well bring about still further nitrate deficiency and decrease in yields, although the suppressing action of even high concentrations of phosphate on nitrate absorption is less common than that of nitrate on phosphate intake.

The writer is indebted to W. A. WENDT and S. C. CHRISTIAN, who made their experiments available for these investigations, and to LAWRENCE QUINN for extensive statistical studies. He appreciates the help of VALDEMAR KNUDSEN in

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LITERATURE CITED

1. ANDERSSEN, F. G., Citrus manuring: its effect on cropping and on the composition and keeping quality of oranges. Jour. Pomol. and Hort. Sci. 15:117–159. 1937.
2. ARNON, D. I., Effect of ammonium and nitrate-nitrogen on the mineral composition and sap characteristics of barley. Soil Sci. 48:295–307. 1939.
3. BATJER, L. P., and DEGMAN, E. S., Effects of varying amounts of nitrogen, potassium, and phosphorus on growth and assimilation of young apple trees. Proc. Amer. Soc. Hort. Sci. 35:255. 1938.
4. BECKENBACH, J. R., ROBBINS, W. R., and SHIVE, J. W., Nutrition studies with corn. 2. A statistical interpretation of the relation between the ionic concentration of the culture solutions and the element content of the tissues. Soil Sci. 45: 403–426. 1938.
5. BURKHART, L., and PAGE, N. R., Mineral nutrient extraction and distribution in the peanut plant. Jour. Amer. Soc. Agron. 33:743–755. 1941.
6. BURSTRÖM, H., Über antagonische Erscheinungen bei der Kationaufnahme des Hafers. Svensk. Bot. Tidskr. 28:157–263. 1934.
7. CAROLUS, R. L., The use of rapid chemical plant nutrient tests in fertilizer deficiency diagnoses and vegetable crop research. Virginia Truck Exp. Sta. Bull. 98. 1938.
8. CHANDLER, R. F., JR., Absorption, distribution, and seasonal movement of potassium in young apple trees and the effect of potassium fertilizers on potassium and nitrogen content and growth of trees. Jour. Agr. Res. 53:19–42. 1936.
9. CHAPMAN, G. W., Leaf-analysis and plant nutrition. Soil Sci. 52:63–81. 1941.
10. CHAPMAN, H. D., Inorganic phosphate in green plant tissue as a measure of phosphate availability. Soil Sci. 39:111–122. 1935.
11. CHAPMAN, H. D., LIEBEG, G. F., JR., and VANSELOW, A. P., Some nutritional relationships as revealed by a study of mineral deficiency and excess symptoms on citrus. Amer. Proc. Soil Sci. Soc. 4:196–200. 1939.
12. CHAPMAN, H. D., and LIEBEG, G. F., JR., Nitrate concentration and ion balance in relation to citrus nutrition. Hilgardia 13:141–173. 1940.
13. DAVIDSON, J., The possible effect of hydrogen-ion concentration on the absorption of potassium and phosphorus by wheat plants under field conditions. Jour. Agr. Res. 46:449–453. 1933.
14. DAVIDSON, O. W., and BLAKE, M. A., Nutrient deficiency and nutrient balance with the peach. Proc. Amer. Soc. Hort. Sci. 35:339–346. 1937.
15. DIKUSSAR, I. G., The physiological significance of ammonium salts in relation to changes in the composition of the nutrient solution (transl. title). Lenin Acad. Agr. Sci., Gedroiz Inst. Fert. Agro. Soil Sci. 3:67–76. 1934.
16. FONDER, J. F., Variations in potassium content of alfalfa due to stage of growth and soil type and the relationship of potassium and calcium in plants grown upon different soil types. Jour. Amer. Soc. Agron. 21:732–750. 1929.
17. GARNER, W. W., MCMURTREY, J. E., JR., BOWLING, J. D., JR., and MOSS, E. G., Magnesium and calcium requirements of the tobacco crop. Jour. Agr. Res. 40:145–168. 1930.
18. GREGORY, F. G., Mineral nutrition of plants. Ann. Rev. Biochem. 6:557–578. 1937.
19. HAAS, A. R. C., and REED, H. S., The absorption of ions by citrus and walnut seedlings. Hilgardia 2:67–106. 1926.
20. HAMNER, K. C., LYON, C. B., and HAMNER, C. L., Effect of mineral nutrition on the ascorbic-acid content of the tomato. BOT. GAZ. 103:586–616. 1942.
21. HEPLER, J. R., and KRAYBILL, H. R., Effect of phosphorus upon the yield and time of maturity of the tomato. New Hampshire Agr. Exp. Sta. Tech. Bull. 28. 1925.
22. HOAGLAND, D. R., Absorption of mineral elements by plants in relation to soil problems. Plant Physiol. 6:373–388. 1931.
23. HOAGLAND, D. R., and ARNON, D. I., Physiological aspect of availability of nutrients for plant growth. Soil Sci. 51:431–444. 1941.
24. JANSSEN, G., and BARTHOLOMEW, R. P., The translocation of potassium in tomato plants and its relation to their carbohydrate and nitrogen distribution. Jour. Agr. Res. 38:447–465. 1929.
25. KRAUS, E. J., and KRAYBILL, H. R., Vegetation and reproduction with special reference to the tomato. Oregon Agr. Exp. Sta. Bull. 149. 1918.
26. KYZLINK, V., Variations in the potassium content of flax plant ash in the light of the newer biochemical knowledge. Boden. Pflanzen. 14: 196–204. 1939.
27. LAUDE, H. M., Combined effects of potassium supply and growth substances on plant development. BOT. GAZ. 103:155–167. 1941.
28. LILLELAND, O., and BROWN, J. G., The phosphate nutrition of fruit trees. 2. Continued response to phosphate applied at the time of planting. Proc. Amer. Soc. Hort. Sci. 37:53–57. 1939.

29. LUNDEGÅRDH, H., The influence of the soil upon the growth of the plant. Soil Sci. 40:89–101. 1935.
30. MARSH, R. S., Preliminary studies on the sulphur content of the tomato. Proc. Amer. Soc. Hort. Sci. 19:83–84. 1922.
31. MAYNARD, L. A., Relation of soil and plant deficiencies and of toxic constituents in soils to animal nutrition. Ann. Rev. Biochem. 10:449–470. 1941.
32. MCCALLA, A. G., and WOODFORD, E. K., The effect of potassium supply on the composition and quality of wheat. II. Canad. Jour. Res. 13: 339–354. 1935.
33. ———, and ———, Effects of a limiting element on the absorption of individual elements and on the anion:cation balance in wheat. Plant Physiol. 13:695–712. 1938.
34. MCMURTREY, J. E., Symptoms on field-grown tobacco characteristic of the deficient supply of each of several essential chemical elements. U.S.D.A. Tech. Bull. 612. 1938.
35. NIGHTINGALE, G. T., The biochemistry of the nitrogenous constituents of the green plants. Ann. Rev. Biochem. 5:513–524. 1936.
36. ———, The nitrogen nutrition of green plants. Bot. Rev. 3:85–174. 1937.
37. ———, Nitrate and carbohydrate reserves in relation to nitrogen nutrition of pineapple. BOT. GAZ. 103:409–456. 1942.
38. NIGHTINGALE, G. T., SCHERMERHORN, L. G., and ROBBINS, W. R., Some effects of potassium deficiency on the histological structure and nitrogenous and carbohydrate constituents of plants. New Jersey Agr. Exp. Sta. Bull. 499. 1930.
39. NIGHTINGALE, G. T., and FARNHAM, R. B., Effects of nutrient concentration on anatomy, metabolism, and bud abscission of sweet pea. BOT. GAZ. 97:477–517. 1936.
40. PHILLIS, E., and MASON, T. G., Further studies on transport in the cotton plant. 7. Simultaneous changes in the production and distribution of dry matter under varying potassium supply. Ann. Bot. N.S. 3:889–899. 1939.
41. REED, H. S., and HAAS, A. R. C., Studies on the effects of sodium, potassium, and calcium on young orange trees. Univ. California Publ. Agr. Exp. Sta. Coll. Agr. Tech. Paper 11. 1923.
42. RUSSELL, E. J., Soil conditions and plant growth. 7th ed. Longmans. 1937.
43. SHIVE, J. W., The balance of ions and oxygen tension in nutrient substrates for plants. Soil Sci. 51:445–459. 1941.
44. SHIVE, J. W., and ROBBINS, W. R., Mineral nutrition of plants. Ann. Rev. Biochem. 8:503–520. 1939.
45. SHULL, C. A., Phosphorus and the early maturity of plants. Trans. Illinois Acad. Sci. 1925: 165–171. 1926.
46. SOMMER, A. L., Mineral nutrition of plants. Ann. Rev. Biochem. 10:471–490. 1941.
47. SIDERIS, C. P., KRAUSS, B. H., and YOUNG, H. Y., Assimilation of ammonium and nitrate by pineapple plants grown in nutrient solutions and its effects on nitrogenous and carbohydrate constituents. Plant Physiol. 13:489–527. 1938.
48. ———, ———, and ———, Distribution of different nitrogen fractions, sugar, and other substances in various sections of the pineapple plant grown in soil cultures and receiving either ammonium or nitrate salts. Plant Physiol. 14:227–254. 1939.
49. STEWARD, F. C., Mineral nutrition of plants, Ann. Rev. Biochem. 4:519–544. 1935.
50. STREBEYKO, P., The influence of phosphorus on the growth of oats and on the intake of mineral compounds (P, N, S) at different periods of vegetation. (English summary.) Acta Soc. Bot. Poloniae 11:213–237. 1934.
51. THOMAS, W., and MACK, W. B., Foliar diagnosis of differentially fertilized greenhouse tomatoes with and without manure. Jour. Agr. Res. 60: 811–832. 1940.
52. ———, and ———, A foliar diagnosis study of the nutrition of greenhouse tomatoes in relation to the incidence of a disease. Pennsylvania Sta. Bull. 405. 1941.
53. ———, and ———, Foliar diagnosis in relation to soil heterogeneity. Soil Sci. 52:455–468. 1941.
54. TIEDJENS, V. A., and WALL, M. E., The importance of potassium in the growth of vegetable plants. Proc. Amer. Soc. Hort. Sci. 36:740–743. 1938.
55. VOTH, P. D., and HAMNER, K. C., Responses of *Marchantia polymorpha* to nutrient supply and photoperiod. BOT. GAZ. 102:169–205. 1940.
56. WALL, M. E., The role of potassium in plants. 3. Nitrogen and carbohydrate metabolism in potassium deficient plants supplied with either nitrate or ammonium nitrogen. Soil Sci. 49:393–408. 1940.
57. WATSON, R., and PETRIE, A. H. K., Physiological ontogeny in the tobacco plant. 4. The drift in nitrogen content of the parts in relation to phosphorus supply and topping, with an analysis of the determination of ontogenetic changes. Australian Jour. Exp. Biol. Med. Sci. 18:313–340. 1940.
58. WILLIAMS, R. F., Physiological ontogeny in plants and its relation to nutrition. 2. The effect of phosphorus supply on the growth of the plant and its parts. Australian Jour. Exp. Biol. Med. Sci. 14:165–185. 1936.
59. ———, Physiological ontogeny in plants and its relation to nutrition. 4. The effect of phosphorus supply on the total-, protein-, and soluble-nitrogen contents, and water content of the leaves and other plant parts. Australian Jour. Exp. Biol. Med. Sci. 16:65–83. 1938.
60. WOODFORD, E. K., and MCCALLA, A. G., The absorption of nutrients by two varieties of wheat grown on the black and gray soils of Alberta. Canadian Jour. Res. Sec. C 14:245–266. 1936.

GROWTH SUBSTANCES AND DORMANCY OF SPORES OF PHYCOMYCES

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(WITH FOUR FIGURES)

Introduction

In earlier papers from this laboratory the low percentage germination of spores of *Phycomyces blakesleeanus* at 25° or 26° C.[1] on an agar medium containing minerals, sugar, asparagine, and thiamine was reported (4, 5, 6). The addition to this medium of extracts of various natural products (for example, molasses or extracts of potato tubers) increased germination from 0.5 per cent or less on the basal medium to more than 90 per cent on the medium containing the natural product. Extracts of potato tubers were fractioned by treatment with charcoal. The charcoal eluate (Ca fraction) was somewhat effective in increasing germination, and so was the filtrate (D_R fraction), but the two fractions acting together produced more than an additive effect. Two growth substances were assumed to be present and to account for the effect of the extracts. One of these, factor Z_1, was adsorbed on charcoal; the other, factor Z_2, was present in the filtrate from the charcoal-treated extracts (6). Both were thermostable, and neither was identical with any of the known vitamins, amino acids, or other growth substances tested (5, 6). Factor Z_1 appeared to be hypoxanthine (7, 8). Factor Z_2 remains unidentified (2, 3, 4).

It seems, therefore, that the small percentage germination at 25°–26° on a mineral-dextrose medium containing asparagine and thiamine is not because of lack of viable spores of *Phycomyces*, but because on such a medium almost all the spores are dormant. Their dormancy can be broken by the presence of extracts of various natural products.

We have investigated the dormancy of these spores because of interest in the Z factors, which influence not only spore germination but growth and gametic reproduction as well. The problem is of interest also because of the similarity between the effect of the Z factors on *Phycomyces* and that of factors which have been studied by some investigators for other microörganisms (1, 10). There is the possibility that the phenomena concerned in the dormancy of the spores may be similar to those determining dormancy in other organisms, including some of the higher plants.

Material and methods

A plus strain of *Phycomyces blakesleeanus* was used throughout the investigation. Unless otherwise stated, stock cultures were grown at 20° in the dark on the basal mineral-dextrose medium containing asparagine, thiamine, and 1.5 per cent Difco agar. Sporangiophores and sporangia were pulled from these cultures with a platinum hook and shaken in distilled water. A loop, 3 mm. in diameter, was used to place a drop of the spore suspension on the surface of 10 ml.

[1] Temperatures throughout this paper are Centigrade.

of the basal medium, to which 1 per cent purified agar had been added, in a petri dish 15 × 90 mm. The dishes were incubated in the dark at 26°–26.5°, and spore counts were made under the low power of a compound microscope after 3–18 hours, depending on the rapidity of germination. As a rule, 100 spores were counted in a single drop. A hand counter helped in recording their numbers. No attempt was made to determine probable errors, as the effects studied were usually sufficiently large and consistent to obviate the necessity of considering them statistically. Most experiments were repeated, many of them several times.

Although our primary interest was in percentage germination, observations were made in many experiments on the growth of the germ tubes and on the diameter and thickness of the mycelial colonies which developed. Some experiments were performed in liquid culture, in which the dry weights of the mycelium were determined.

The basal medium contained per liter: 50 gm. dextrose, 1.5 gm. KH_2PO_4, 0.5 gm. $MgSO_4 \cdot 7H_2O$, 2 gm. asparagine, and 500 μgm. thiamine. To this medium minor elements were added in p.p.m.: 0.005 B, 0.02 Cu, 0.1 Fe, 0.01 Ga, 0.01 Mn, 0.01 Mo, and 0.09 Zn.

All glassware was cleansed with chromic-sulphuric acid cleaning mixture and thoroughly rinsed with tap and distilled water.

Experiments

PERSISTENCE OF DORMANT CONDITION.—At 26° on the basal agar medium, normal untreated spores of *Phycomyces* remained dormant for several days and probably for a month or more. For example, spores from cultures grown 11 days at 20° showed no germination on the basal medium after 24 hours at 26°. On an agar containing potato extract[2] the germination was 98 per cent. The spores on the basal medium showed no germination after 1 week.

CHANGES IN DORMANCY WITH AGE.—Young spores, 1–2 weeks old, showed little or no germination at 26° on the basal medium; a thousand or more spores might be examined before one was found germinating. The percentage germination from progressively older cultures increased to a maximum and then declined. In one experiment spores 1 month old showed 2 per cent germination; this increased to 12 per cent for spores $2\frac{1}{2}$ months old. After 5 months the germination was 4 per cent, and after $8\frac{1}{2}$ months it was less than 1 per cent. A few spores germinated (less than 1 per cent) from cultures 317 days old (table 11).

The lower percentage germination of the younger spores was a dormancy phenomenon, because on agar containing potato extract the germination was nearly 100 per cent. As the spores aged viability decreased. In one experiment spores 4–77 days old germinated 80 per cent or more on the potato-extract agar, but spores 155 days old showed on the same medium but 17 per cent germination, and spores 317 days old showed but 1 per cent (table 11). It seems probable that as the spores grew older changes occurred which broke dormancy; at the same time some spores, probably those ready to germinate, died, until few viable spores—either dormant or active—remained after 317 days. The rapidity of germination changed with age. Spores older than 2 months were slower in germinating than the younger ones.

[2] The potato extract was prepared as described earlier (6). The extract used here contained 500 mg. dry matter per millimeter, and 0.1 ml. was used per petri dish.

Because of the effect of age on germination, spores less than 2 weeks old were customarily used in these experiments.

MINOR FACTORS AFFECTING GERMINATION.—The temperature at which the spores developed had some, though little, effect on the percentage germination. Spores grown at 26° behaved as though they were considerably older than those grown at 20° or lower. Spores from cultures grown 11 days at 26° germinated 3 per cent; spores from cultures of the same age grown at 11° or 20° showed no germination. The tests were carried out at 26° on the basal medium.

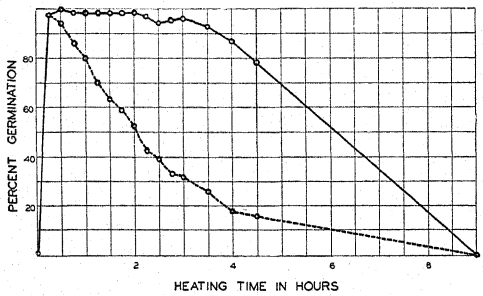


FIG. 1.—Time of heating spores of *Phycomyces* at 50° C. and percentage germination on basal agar at 26° C. Solid line, grown in dark; dotted line, grown in diffuse light.

Spores from cultures grown on the basal medium in the light at 25° for 7 or 14 days germinated a little better than those grown in the dark. The maximum germination observed for 14-day-old spores, grown in faint diffuse light, was 2.3 per cent. However, spores which developed in the light were more easily injured at 50° than were those which developed in the dark. This difference is illustrated by the following experiment. Cultures were grown 8 days in a light-tight box at 25°. By the side of this box other cultures were grown in weak diffuse light. A spore suspension from each set of cultures was then exposed at 50° in a water-bath, and at various times spores were removed and germinated at 26° on the basal medium. The spores grown in the light gave maximum germination after 15 minutes' treatment and progressively lower germination with longer exposures (fig. 1). The spores grown in the dark showed maximum germination with 15 minutes' exposure to heat and little decrease until the treatment exceeded $3\frac{1}{2}$ hours. With 2 hours' exposure at 50° the spores grown in the light germinated 52 per cent; those grown in the dark, 99 per cent.

Light appeared to exert its effect in the early stages of development. Spores grown for 1 week or 10 days in the dark, and then exposed to light for several days, responded as did spores which had been in the dark continuously. There was some indication also that the differences between spores grown in the light and in the dark in susceptibility to heat may disappear as the spores age.

The causes for this difference are obscure. They may be entirely physiological or related in part to the structure of the spores. Spores from cultures grown at 25° in weak diffuse light were smaller, more elongated, and thinner walled; those which developed in the dark were larger, rounder, and thicker walled.

In any event, these observations emphasized the need to consider the light conditions in studies on spore germination of *Phycomyces*, and spores grown at 20° in a dark cabinet were used as standard material. These spores were not in complete darkness. A little light reached them when the cabinet was opened, but this light was insufficient to induce a phototropic response of the sporangiophores.

In contrast to the minor influences of age, light, and the temperature under which the spores had developed, other factors were found to exert a major effect on dormancy.

HYPOXANTHINE AND THE D_R FRACTION.—Both hypoxanthine and the D_R fraction[3] (containing the Z_2 factor) increased the number of spores germinating at 26°. When both factors were present the effect was greater than that produced by the same quantity of either alone. Their action, however, was not entirely specific. There seemed to be something analogous to a mass action effect; that is, a large amount of either hypoxanthine or the D_R fraction (factor Z_2) was effective, and a large amount of the D_R fraction plus a small amount of hypoxanthine had the same effect on germination as a small amount of the fraction and a large amount of hypoxanthine. Although both factors favorably influenced germination and growth, hypoxanthine appeared to be primarily a germination factor, while the D_R fraction had the greater effect on growth. These general statements are illustrated by the following experiment.

Spore suspensions were prepared from cultures grown at 20° which were 8 days and 11 days old, respectively. These suspensions were placed on the basal agar medium and on the same medium to which were added various quantities of hypoxanthine and of a D_R fraction from white potatoes. The spores were germinated at 26°, and colony diameters were measured after 31 hours. In general, the 11-day-old spores showed a greater response to the supplements than those 8 days old. For both lots 100 mμ moles of hypoxanthine[4] were required to affect germination materially. For the older spores 10 mg. of the D_R fraction had some effect, but 100 mg. had more. One mg. of the fraction was effective in combination with 10 or with 100 mμ moles of hypoxanthine; 1 mμ mole of hypoxanthine combined with 10 mg. of the fraction exerted a greater effect than these amounts of either alone. Better growth was obtained with 10 or 100 mg. of the D_R than with 100 mμ moles of hypoxanthine. Best growth occurred when 100 mμ moles of hypoxanthine and 100 mg. of the fraction were present (table 1).

TABLE 1

EFFECT OF VARIOUS AMOUNTS OF HYPOXANTHINE AND $_R$ FRACTION ON PERCENTAGE GERMINATION OF SPORES OF PHYCOMYCES AND LATER COLONY GROWTH. SPORES 11 DAYS OLD. COLONY DIAMETERS MEASURED AFTER 31 HOURS. TEMPERATURE OF INCUBATION 26° C.

ADDITIONS TO 10 ML. BASAL AGAR MEDIUM	NONE	1 Mμ MOLE HYPOZANTHINE	10 Mμ MOLES HYPOZANTHINE	100 Mμ MOLES HYPOZANTHINE
	Germination (%)			
None	0	0.1	0.1	7
0.1 mg. D_R fraction	0	0	0.1	10
1 mg. D_R fraction	0	0	2	12
10 mg. D_R fraction	7	5	29	48
100 mg. D_R fraction	49	77	98	98
	Colony diameter (mm.)			
None	0*	0*	7‡	8‡
0.1 mg. D_R fraction	0*	0*	6‡	9‡
1 mg. D_R fraction	0*	0*	11‡	10‡
10 mg. D_R fraction	15†	10	15	20
100 mg. D_R fraction	22	24	27	30

* Germination none or almost none. † Thin. ‡ Very thin.

Our observations have led to the assumption that the spores are dormant because of the lack within them of a sufficient amount of Z factors (hypoxanthine

[3] Preparation of Ca and D_R fractions from potato tubers has been described earlier (6).

[4] Somewhat older spores were more sensitive to hypoxanthine. See later section.

and factor Z_2) in available form. It has further been assumed that when a sufficient amount of the Z factors is supplied by addition to the medium of extracts of potato or other natural products, or of hypoxanthine and the D_R fraction, the spores absorb the factors and germination occurs. It would also seem that, at 26°, actively growing mycelium of *Phycomyces* suffers from a partial deficiency[5] for the Z factors, with the result that their addition to the medium increases the amount of growth. This hypothesis will be considered with reference to various factors which break the dormancy of the spores.

TEMPERATURES ABOVE 26° C.—Early in this investigation it was found that relatively short exposures of a spore suspension to a temperature of 50° ± 1° induced a high percentage of germination on the basal agar medium. No germination of untreated spores occurred at this temperature; in fact, the spores were killed if the treatment was sufficiently long, but spores heated a short time at 50° appeared to be activated in such a way that their germination at 26° was increased to nearly 100 per cent (fig. 1).

One procedure in activating spores by heat was as follows: A test tube containing 5 ml. of a spore suspension in distilled water was placed in a hot-air oven heated to 50°. At various intervals loopfuls of the suspension were removed from the tube and placed on the basal medium for germination at 26°. Exposures up to 30 minutes resulted in progressively increased germination, until nearly 100 per cent was obtained with the 30-minute treatment. Exposures longer than 3 hours and 15 minutes resulted in decreased germination. With this procedure the actual temperature to which the spores were subjected was less than 50° for a considerable part of the shorter periods of heating, because of the time required for the 5 ml. of water, initially at room temperature, to reach 50°. When the water was preheated to 50° before the spores were added, 7.5 minutes were sufficient to produce maximum activation.

In many experiments it was found more convenient to immerse the tubes in a waterbath held at 50° than to set them in a hot-air oven. Furthermore, the temperature control in the waterbath was considerably more accurate than that in the hot-air oven. The procedure usually employed for heat activation was to expose the suspensions for between 30 minutes and 1 hour in a waterbath, starting with cold tubes or flasks.

Some differences were noted among various lots of spores in the time at which reduction of germination occurred. In one experiment the time was 3 hours and 15 minutes; in other instances it was as low as 2 hours and 15 minutes. Spores which developed in the light were quickly injured by exposure at 50°, as described earlier. Even when the longer periods of heating did not cause decrease in spore germination, some reduction in growth of the mycelium was observed. However, exposure of between 30 minutes and 1 hour always gave maximum germination and little or no inhibition of growth.

It is reasonable to expect that shorter periods at higher temperatures and longer periods at lower temperatures than those reported would also activate the spores. No extensive investigation of other temperatures was made. It was found, however, that exposure of a suspension for 2 hours and 10 minutes at 35° was ineffective, while 20 hours gave

[5] By a partial deficiency is meant that the organism makes from simple food materials some of the substance but not enough for maximum growth (9).

nearly maximum germination. All the spores were killed when the suspension was heated for 10 minutes at 60° and when 1 ml. of a suspension was placed for 1 minute in a waterbath heated to 80°.

Treatment of dry spores with heat was much less effective than exposure of the spores suspended in water. No effect on germination was observed when open tubes were exposed at 35° for 20 hours. Ten minutes at 100° was partially effective.

HEAT ACTIVATION AND EARLY MYCELIAL GROWTH.—Heat activation not only influenced germination but materially affected the early growth of the mycelium. This is shown by the following experiment.

Spores 10 days old were activated by heating for 2 hours at 50°. The unactivated spores germinated 0.05 per cent and the activated spores 97 per cent. Some flasks containing 25 ml. of the basal solution, and the same solution supplemented with a Ca fraction, a D_R fraction, or both, were inoculated with the activated and others with the unactivated spores. The flasks were incubated at 26° and dry weights determined after 48 and 72 hours. At the end of 48 hours the activated spores had produced nearly as much mycelium in the basal solution as the unactivated spores produced in the presence of the combined Ca and D_R fractions (table 2). After 72 hours the activated spores had formed in the basal solution nearly as much mycelium as the unactivated spores had in a solution supplemented with the D_R fraction, but considerably less than was obtained in the solution containing both Z factors. These results strongly suggest that heat-activated spores contained more available Z factors than unactivated spores. This follows because the activated spores grew in the absence of the Z factors much as the unactivated spores did in their presence. The activated spores did not contain a sufficient amount of the factors to permit maximum growth, however; addition of Z factors to the medium improved their growth as compared with that in the basal solution.

The influence of the D_R fraction and the combined Ca and D_R fractions was in part independent of their effect on germination. This follows because the D_R fraction and the combined Ca and D_R fractions favorably influenced growth of the activated spores, which germinated 97 per cent.

TABLE 2

GROWTH OF UNACTIVATED AND HEAT-ACTIVATED SPORES IN VARIOUS SOLUTIONS

Additions to 25 ml. basal medium	Dry weight (mg.) of two mycelia after 48 hours in solutions plus		Dry weight (mg.) of two mycelia after 72 hours in solutions plus	
	Unactivated spores	Activated spores	Unactivated spores	Activated spores
None	0.0	8.5	3.6	121.0
0.1 ml. Ca fraction	5.2	9.4	27.2	95.0
0.1 ml. D_R fraction	8.0	25.4	148.0	219.0
Ca fraction and D_R fraction	10.4	26.4	210.0	240.0

PARTIAL ACTIVATION WITH HEAT AND THE Z FACTORS.—As already indicated, heating spores under certain conditions caused maximum germination and increased the growth obtained in the basal solution. These results were interpreted to mean that heating increased the available Z factors in the spores. That heat activation has this effect is supported further by the observation that spores partially activated by heat responded to hypoxanthine and the D_R fraction as

though they contained more of the Z factors than the unactivated spores. For example, in the experiment summarized in table 3, the unactivated spores showed no response to 10 mμ moles of hypoxanthine. The partially activated spores germinated 38 per cent without the hypoxanthine and 95 per cent with it. This could mean that the Z factors in the untreated spores plus that supplied by the hypoxanthine in the medium were insufficient to cause germination; after partial activation the increased Z factors in the spores added to that obtained from the hypoxanthine in the medium allowed 95 per cent germination. The same type of response occurred with the D_R fraction and with the combination of the two Z factors.

TABLE 3

EFFECT OF PARTIAL HEAT ACTIVATION AND Z FACTORS ON PERCENTAGE GERMINATION OF SPORES AND ON COLONY DIAMETERS (MEASURED AFTER 48 HOURS). SPORES 9 DAYS OLD

Additions to 10 ml. basal agar medium	Percentage germination		Colony diameter (mm.)	
	Untreated	Partially activated	Untreated	Partially activated
None.....	0	38	.....	28
10 mμ moles hypoxanthine...	0	95	.....	36
10 mg. D_R fraction.....	1	95	32	48
Hypoxanthine and D_R fraction.....	9	98	43	48

If the assumption that partial heat activation increased the amount of available Z factors in the spores is correct, it must be concluded that the quantity of these factors produced by the treatment was insufficient for maximum germination or maximum growth. This follows because the addition of hypoxanthine and the D_R fraction to the medium increased percentage germination and growth (colony diameters) of the activated spores. In this experiment partial activation was accomplished by heating the spore suspension for 5 minutes at 50°. The colony diameters were measured after 48 hours.

PRESENCE OF Z FACTORS IN SPORES.—If heat activation increases the amount of available Z factors in spores, then potentially available Z factors must be present in the spores. Two methods of investigating this question were followed. In one, spores were digested with sulphuric acid and the effects of the digest on germination determined. In the other, the influence of weighed amounts of killed spores on the growth of *Phycomyces* in various solutions was measured.

Spores grown in the basal liquid medium were removed, washed with distilled water, and centrifuged. Spores amounting to 112.5 mg. were digested at boiling temperature with 8N H_2SO_4. The acid was neutralized with $Ba(OH)_2$, the $BaSO_4$ removed, and the filtrate reduced by evaporation to small volume. The digest was added to the basal agar medium. Spores 7 days old showed no germination on this medium; on the same medium plus the digest germination it was 85 per cent; and on potato-extract agar it was 84 per cent.

In a second experiment spores from liquid cultures were removed, washed with distilled water, centrifuged, and activated by heating for 1 hour at 50°. Spores amounting to 8.7, 0.87, and 0.087 mg. were added to flasks containing 25 ml. of the basal solution, to the basal solution plus the Ca fraction (Z_1 factor), and to the basal solution plus the D_R fraction (Z_2 factor). After sterilization the solutions were inoculated with the spores and incubated at 26°. Dry weights

were determined after 72 hours. In the basal medium 8.7 mg. of spores improved growth four times (table 4), and 0.87 mg. had a small favorable effect. The addition of the spores also had a favorable effect in the solutions containing the Ca and the D_R fractions. Apparently a sufficient amount of activated and autoclaved spores favorably affected the early growth of *Phycomyces;* that is, they had the same effect as the addition of hypoxanthine and the D_R fraction to the basal medium.

TABLE 4

PRESENCE OF Z FACTORS IN SPORES. EFFECT OF SPORES ON 72-HOUR GROWTH IN VARIOUS SOLUTIONS

Quantity of spores added to 25 ml. basal medium	Dry weight (mg.) of mycelium produced in two flasks of basal solution supplemented with			
	Nothing	Ca fraction	D_R fraction	Ca and D_R fractions
None	11.7	42.0	139.0	228.0
0.087 mg.	8.5	47.0	190.0	.....
0.87 mg.	15.0	54.0	162.0	.....
8.7 mg.	52.0	87.0	214.0	.....

EXTRACTS FROM HEATED SPORES.—If heat activation transforms Z factors in the spore from an unavailable to an available form, then more Z factors should be extractable from living activated spores than from unactivated ones. This assumption was tested by comparing the effects on the early growth of *Phycomyces* of extracts made with distilled water at 26° from activated and unactivated spores.

Spores were grown in quantity at 20° on the basal solution with asparagine replaced by ammonium glutamate. After 18 days the spores were removed, washed three times with distilled water, and suspended in 20 ml. of distilled water. Ten ml. of the suspension were heated for 20 minutes at 50°. The unactivated spores germinated 23 per cent; the heat-activated spores, 78 per cent. Both suspensions were allowed to stand for 22 hours at 26°. At the end of the extraction period the spores were removed by centrifuging and discarded. Aliquots of each extract were added to 10-ml. quantities of the basal solution, and to the same solution supplemented with hypoxanthine or with the D_R fraction. The extract of 58 mg. of spores was added to each flask. The flasks were sterilized, inoculated, and incubated for 24 hours at 26°. The extract of the unactivated spores improved growth slightly in all

TABLE 5

PRESENCE OF Z FACTORS IN WATER EXTRACTS OF HEAT-ACTIVATED SPORES. GROWTH OF PHYCOMYCES IN VARIOUS SOLUTIONS TO WHICH EXTRACTS OF UNACTIVATED OR HEAT-ACTIVATED SPORES WERE ADDED

Additions to 10 ml. basal medium	Dry weight (mg.) of two mycelia in 48 hours in liquid plus		
	Nothing	Extract unactivated spores	Extract activated spores
	18-day-old spores		
Nothing	0.0	0.9	6.9
50 mμ moles hypoxanthine	20.4	22.0	19.0
13 mg. D_R fraction	38.4	47.0	68.0
Hypoxanthine and D_R fraction	83.0	.....	.....
	9-day-old spores		
Nothing	.....	0.5	3.3
100 mμ moles hypoxanthine	.....	11.8	31.3
13 mg. D_R fraction	.....	31.5	76.8

three solutions, but the extract from the activated spores was markedly effective in the basal solution and in that supplemented with the D_R fraction (table 5).

Since in this experiment the untreated spores showed considerable germination, indicating partial activation somewhere in the process of their preparation, the experiment was in part repeated, using 9-day-old spores. A suspension of 168 mg. of spores in 25 ml. of water was heated for 1 hour at 50°. An equal weight of untreated spores was suspended in 25 ml. of distilled water. The unactivated spores germinated 0.5 per cent; the activated, 96 per cent. Both suspensions were allowed to stand for 24 hours at 26°. The spores were then centrifuged off and discarded and the extracts evaporated to 12 ml. Aliquots of each extract were added to 10-ml. quantities of the basal solution and to the same solution plus hypoxanthine or the D_R fraction. Each flask received the extract of 28 mg. of spores. After sterilization the flasks were inoculated and incubated at 26° for 48 hours. The extracts from the activated spores had a distinctly greater effect on the early growth of *Phycomyces* than had those from the unactivated spores (table 5).

LOSS OF VITALITY OF HEAT-ACTIVATED SPORES.—If the Z factors diffuse into distilled water more rapidly from heat-activated spores than from unactivated, heat-activated spores might be expected to lose their vitality more rapidly when suspended in distilled water than those not so activated. This assumption was confirmed by the following experiment.

A suspension of spores 16 days old was made in 10 ml. of distilled water. Half the suspension was heated for 1 hour at 50°. Both suspensions were placed at 26° and germination determined on potato-extract agar at weekly intervals. At the end of 5 days the unactivated spores germinated 98 per cent; the activated, 88 per cent. After 18 days the unactivated spores germinated 77 per cent; the activated, 10 per cent. After 32 days the unactivated spores germinated 76 per cent; the activated, 1 per cent.

EFFECT OF TEMPERATURES BELOW 26°.—Not only may dormancy be broken by suitable treatment with temperatures above 26°, but the spores respond also to short exposures below 26° under certain conditions, as illustrated by the following experiment.

TABLE 6

TWO-HOUR EXPOSURE ON BASAL AGAR MEDIUM AT TEMPERATURES INDICATED AND PERCENTAGE GERMINATION AT 26° C.

EXPOSURE ON BASAL AGAR MEDIUM (°C.)	PERCENTAGE GERMINATION OF SPORES			
	7 days	10 days	14 days	17 days
3.....	0.5	8	0.9	5
11.....	15	10	7.5	12
15.....	10	19	5	16
20.....	5	4	0.5	1
26.....	0	0.1	0.1	0.1
35.....	.....	0	0.1	0.1

Spores grown at 20° in the dark were placed on the basal agar medium. The petri dishes were exposed for 2 hours at 3°, 11°, 15°, 20°, 26°, or 35° and then transferred to 26° for germination. Spores were treated in this fashion when they were 7, 10, 14, and 17 days old. The spores exposed at 26° and at 35° for 2 hours germinated 0–0.1 per cent; at 20°, 0.5–5 per cent; at 15°, 5.0–19.0 per cent; at 11°, 7.5–15 per cent; and at 3°, 0.5–8.0 per cent (table 6). It is probable that some of the variations in the effect of a given temperature on spores of different ages was because the petri dishes and agar were not precooled but were at

room temperature before the 2-hour treatment.

At any rate, numerous experiments showed that 2 hours' exposure of spores at 11° or 15° on the basal agar medium broke dormancy to a considerable extent;[6] 20° was less effective; and 3° had little effect. Had the plates been precooled, 3° would perhaps have had no effect. Exposures at 26° and 35° were ineffective (table 6).

There was one noteworthy difference between the action at 50° and of temperatures below 26°. Spores suspended in distilled water were activated at 50° but not by the lower temperatures, which were effective only when the spores were exposed on the basal agar medium or in the basal nutrient solution. The ineffectiveness of exposure at 3° as compared with that at 11° or 15° suggests that the action of the favorable lower temperature is associated in some way with metabolism of the spore. This assumption is supported by the fact that the lower temperatures were not effective unless the components of a full nutrient solution were available during the exposure.

If the presence of sufficient amounts of available Z factors is necessary for germination, as assumed, then it must also be assumed that metabolism of the spores for 2 hours at 11° or 15° produces more available Z factors than at 26°. Since the rate of metabolism is doubtless more rapid at 26° than at 11°, apparently the kind of metabolism at 11° or 15° differs from that at 26° and results in more rapid accumulation of available Z factors.

The temperature at which spores were grown seemed to have little effect on the percentage germination on the basal agar medium, although there was considerable difference in the response to the Z factors and to short exposure to low temperature.

Spores 11 days old, grown at 11°, 20°, and 26°, were germinated on the basal agar medium and on the same medium supplemented with the Z factors (table 7). Petri dishes containing spores grown at each of these temperatures were exposed for 2 hours at 11° and then placed at 26° for germination. In general, the response of the spores to the Z factors and to exposure to low temperature decreased with the temperature at which they were grown. Spores grown at 11° showed the greatest response to the Z factors; those grown at 26° benefited less from short exposure to 11° than those grown at 11°. Spores grown at 11° appeared to contain a greater quantity of the Z factors which could be made available by exposure at 11°.

The response to hypoxanthine of the 20° and 26° spores exposed 2 hours at 11° was great, suggesting that much of the

TABLE 7

EFFECT OF SHORT EXPOSURE AT 11° C. ON SPORES GROWN AT VARIOUS TEMPERATURES. SPORES 11 DAYS OLD

Additions to 10 ml. basal agar medium	Percentage germination at 26° C.					
	Of spores grown at			After 2 hours at 11° C. of spores grown at		
	11°	20°	26°	11°	20°	26°
None	0	0	3	93	39	17
100 mμ moles hypoxanthine	41	7	16	96	77	62
100 mg. D_R fraction	84	49	24	94	89	90
100 mμ moles hypoxanthine and D_R fraction	98	98	89	98	99	86

[6] Exposure of $2\frac{1}{2}$ hours at 11° on precooled agar media gave 50 per cent germination when the check showed 0.1 per cent.

Z_2 factor had accumulated at the lower temperature.

ACETATE.—The action of these various physical factors can be duplicated in part by chemicals, which also may be assumed to affect the availability of Z factors. The addition of 10 mg. acetate (prepared from acetic acid redistilled from glass) as sodium acetate per petri dish containing 10 ml. of the basal medium produced maximum germination; 1 mg. was partially effective.

Spores germinated also after soaking in the basal nutrient solution containing acetate. They were suspended for 3 hours at 26° in 10 ml. of the basal solution containing 10 mg. acetate as sodium acetate and then placed on the basal agar medium. Swollen spores were observed within 30 minutes, and maximum germination occurred; but treatment in distilled water containing acetate for 2–26 hours was ineffective. An attempt was made to define the portion of the nutrient solution concerned in promoting the acetate effect. The action of acetate in a solution of the minerals of the basal solution and thiamine was slight (8 per cent germination); it was somewhat greater in a solution of dextrose, asparagine, and thiamine (40 per cent); with the complete nutrient solution it was 99 per cent. Apparently the activity of acetate in breaking dormancy was associated in some way with the presence of the constituents of a complete nutrient solution.

EFFECT OF OTHER ORGANIC ACIDS.—The effect of the sodium salts of other organic acids[7] also was investigated. One mg. of each acid in the form of its sodium salt was added to a petri dish containing 10 ml. of the basal agar medium. This was repeated, using 10 mg. of each acid per petri dish. The acids fell in the following order: propionate > acetate > pyruvate > *n*-butyrate > *n*-valerate > *iso*-butyrate, formate. The propionate at 10 mg. gave maximum germination but reduced growth materially; at 1 mg. it was superior to acetate in its effect on germination and had little or no effect on growth. The pyruvate at 10 mg. materially increased germination but also completely inhibited growth; at 1 mg. it had no effect on germination. The *n*-butyrate at 10 mg. increased germination but was ineffective at 1 mg. The *n*-valerate at 10 mg. inhibited germination but was slightly active at 1 mg. *Iso*-butyrate and formate were slightly effective at 10 mg. and ineffective at 1 mg. The citrate, diethylacetate, fumarate, lactate, malate, malonate, oxalate, succinate, and tartrate were ineffective. The *n*-caproate at 10 mg. completely inhibited germination and was ineffective at 1 mg.

RELATION BETWEEN ACETATE AND Z FACTORS.—If acetate breaks dormancy of the spores by releasing Z factors already present but in unavailable form, the effects of acetate and of the Z factors should be expected to supplement each other. This appeared to be true. The germination of spores partially activated by acetate was improved by the presence of hypoxanthine or the D_R fraction. In one experiment spores 13 and 35 days old were placed on the agars indicated (table 8). In general, the older spores gave the greater responses. On the basal agar medium no germination of the spores 13 days old occurred. The addition of 1 mg. acetate increased germination to 13 per cent. Hypoxanthine and 1 mg. acetate gave 71 per cent, although germination with hypoxanthine alone was 7 per cent. Germination with the D_R fraction alone was 9 per cent, and with

[7] With the exception of the acetate, the acids used were obtained from Eastman Kodak Company and were not especially purified.

the D_R fraction and 1 mg. acetate it was 76 per cent. Maximum germination was obtained on the basal medium to which 10 mg. acetate were added. The Z factors affected the mycelial growth as measured by colony diameters, even in the presence of 10 mg. acetate, where complete germination occurred throughout. Since 10 mg. acetate approaches the maximum which may be used without reducing growth, it would appear that acetate was not a complete substitute for the Z factors.

TABLE 8

EFFECT OF ACETATE AND Z FACTORS ON GERMINATION AND COLONY DIAMETERS OF PHYCOMYCES. SPORES 13 DAYS OLD

Addition to 10 ml. basal agar medium	Percentage germination of spores on basal medium plus			Colony diameters (mm.) after 28 hours on basal medium plus		
	Nothing	1 mg. acetate	10 mg. acetate	Nothing	1 mg. acetate	10 mg. acetate
None	0	13	97	.....	5	12
10 mμ moles hypoxanthine	7	71	97	9	12	15
13 mg. D_R fraction	9	76	97	18	19	20
Hypoxanthine and D_R fraction	64	99	99	23	23	23
0.1 ml. potato extract	93	.....	.....	30	.....	.....

PYRIDINE.—Spores soaked in 5 per cent aqueous pyridine for 70 hours at about 3° gave high percentage germination. Treatment for 24 hours at 26° with 5 per cent pyridine killed most of the spores; 2.5 per cent gave 74 per cent germination; 1.25 per cent gave 12 per cent germination; 0.625 per cent gave 2 per cent; and water gave less than 0.5 per cent. The effect of the pyridine increased with the time of treatment. For spores which showed less than 0.5 per cent germination, 2 hours at 26° with 2.5 per cent pyridine gave 7 per cent germination; 6 hours, 24 per cent.

RELATION OF PYRIDINE, ACETATE, AND THE Z FACTORS.—The combined action of pyridine and acetate seemed to be similar to that of the Z factors. Spores 16 days old were suspended in water and in 0.625 and 1.25 per cent pyridine for 24 hours at 26°. They were then placed on the basal medium and on the same medium plus acetate and the Z factors. On the basal medium (table 9) germination was 0.5 per cent; acetate increased it to 38 per cent; acetate and 0.625 per cent pyridine to 73 per cent; and acetate and 1.25 per cent pyridine to 97 per cent. Germination on the agar containing hy-

TABLE 9

EFFECT OF PYRIDINE, ACETATE, AND Z FACTORS ON GERMINATION OF 16-DAY-OLD SPORES OF PHYCOMYCES

Addition to 10 ml. basal agar medium	Percentage germination after 24 hours					
	In water		In 0.625 pyridine		In 1.25 pyridine	
	No acetate	1 mg. acetate	No acetate	1 mg. acetate	No acetate	1 mg. acetate
None	0.5	38	2	73	12	97
10 mμ moles hypoxanthine	6	88	18	96	49	99
13 mg. D_R fraction	5	80	30	92	65	99
10 mμ moles hypoxanthine and 13 mg. D_R	60	94	74	99	97	99
100 mμ moles hypoxanthine	12	.....	27	.....	68	.....
100 mμ moles hypoxanthine and 13 mg. D_R	47	.....	67	.....	94	.....

poxanthine was 6 per cent; hypoxanthine and acetate, 88 per cent; and for spores treated with 0.625 per cent pyridine and germinated on the agar containing both hypoxanthine and acetate it was 96 per cent. However, growth (as measured by colony diameters) of those spores treated with 1.25 per cent pyridine and grown on the acetate agar was favorably influenced by the Z factors (table 10). This effect was independent of germination, since these spores germinated completely on all media. This suggests that although pyridine and acetate have the same kind of effect on the spores as the Z factors, their effect is not great enough to produce maximum growth. This is to be expected. The quantity of Z factors released in a single spore must of necessity be small. If the factors are used in growth, the quantity in a single spore must be quickly exhausted.

TABLE 10

EFFECT OF PYRIDINE, ACETATE, AND Z FACTORS ON COLONY DIAMETERS (CF. TABLE 9)

ADDITION TO 10 ML. BASAL AGAR MEDIUM	COLONY DIAMETER AFTER 24 HOURS					
	In water		In 0.625 pyridine		In 1.25 pyridine	
	No acetate	1 mg. acetate	No acetate	1 mg. acetate	No acetate	1 mg. acetate
None	6*	11	6*	9	6*	8
10 mμ moles hypoxanthine	13*	12	12	13	14	11
13 mg. D_R fraction	18	24	19	23	19	22
10 mμ moles hypoxanthine and 13 mg. D_R	25	26	22	24	24	24
100 mμ moles hypoxanthine	24	.....	14	.....	12	.....
100 mμ moles hypoxanthine and 13 mg. D_R	24	.....	23	.....	20	.....

* Very thin.

WATER.—Spores showed relatively little change in dormancy for some months if left in the sporangia. However, 2 weeks' immersion in distilled water at 26° broke the dormancy for a considerable percentage of them, and 4 weeks was still more effective. This is illustrated by the following experiment.

Spores grown at 20° for 8, 30, 78, 113, and 264 days were suspended in distilled water and the tubes set in an incubator at 26°. Spores were transferred to the basal agar medium and to potato-extract agar when the experiment was initiated and after 14 and 28 days. On the basal medium the 8-day spores showed no germination at the start, 48 per cent after 14 days, and 81 per cent after 28 days. On the potato-extract agar germination was 96, 99, and 93 per cent. Germination of the older spores also was increased by soaking but was less after 28 days than after 14 days.

AGE, ACETATE, AND THE Z FACTORS.—Spores of various ages were germinated on the basal agar medium, and on the same medium to which 1 mg. acetate, 10 mg. acetate, 10 mμ moles hypoxanthine, 10 mg. D_R fraction, or 0.1 ml. extract of potato tubers was added separately and in combination.

Germination on the potato-extract agar and on the acetate agar showed that the percentage of viable spores decreased with age. The decrease was relatively small up to 77 days and rapid between 77 and 155 days (table 11). The percentage of dormant spores decreased as the spores grew older; the apparent decrease in the proportion of dormant spores between 77 and 255 days was due to the decrease in the percentage of viable spores. The proportion of dormant spores stimulated to

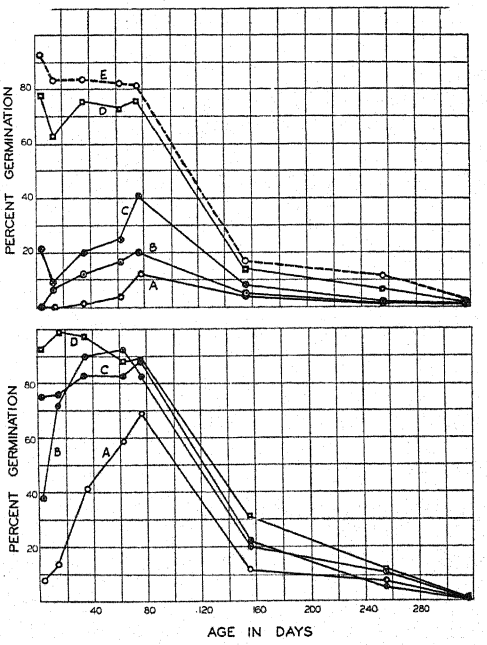
germinate by hypoxanthine or the D_R fraction increased with age. There appeared to be a slow accumulation of

TABLE 11

RELATION OF AGE, ACETATE, AND Z FACTORS TO GERMINATION OF SPORES OF PHYCOMYCES AT 26° C.

Age of spores (days)	Percentage germination on basal agar medium plus				
	Nothing	Hypoxanthine	D_R fraction	Hypoxanthine and D_R fraction	Potato extract
4.....	0	0	22	78	93
13.....	0	7	9	64	84
35.....	2	11	20	76	84
61.....	4	17	25	73	82
77.....	12	20	41	76	81
155.....	4	5	8	14	17
255.....	Less than 1	4	4	7	11
317.....	Less than 1	Less than 1	Less than 1	Less than 1	1
	Plus 1 mg. acetate				
4.....	7	38	75	93	.....
13.....	13	71	76	99	.....
35.....	41	90	83	97	.....
61.....	59	93	83	88	.....
77.....	69	83	88	89	.....
155.....	12	20	22	31	.....
255.....	8	11	6	13	.....
317.....	Less than 1	Less than 1	Less than 1	Less than 1	.....
	Plus 10 mg. acetate				
4.....	93	97	95	95	.....
13.....	97	97	97	99	.....
35.....	99	98	97	99	.....
61.....	94	93	96	94	.....
77.....	86	91	85	87	.....
155.....	24	15	20	24	.....
255.....	10	10	10	11	.....
317.....	Less than 1	1	Less than 1	1	.....

available Z factors in the spores as they grew older, with the result that larger and larger proportions of them germinated on the basal agar medium and were induced to germinate by the presence of one of the Z factors in the agar (table 11; fig. 2). By supplying both the Z factors in the medium it was possible to induce a greater proportion of the spores to germinate than could be accom-



FIGS. 2, 3.—Fig. 2 (above), percentage germination of spores of various ages on various media. *A*, on basal medium; *B*, plus hypoxanthine; *C*, plus D_R fraction; *D*, plus hypoxanthine and D_R fraction; *E*, on potato-extract agar. Fig. 3 (below), percentage germination of spores of various ages on various media, all containing 1 mg. acetate as sodium acetate per petri plate with 10 ml. medium. *A*, basal medium; *B*, plus hypoxanthine; *C*, plus D_R fraction; *D*, plus hypoxanthine and D_R fraction.

plished by furnishing only one. This is interpreted to mean that both factors slowly became available, but at any time the amount present in many spores was below the threshold necessary for germination and required supplementing through a supply in the medium. Comparison of the germination in the presence of acetate and that in the presence of the two Z factors, or of potato extract,

shows that insufficient amounts of these materials were used to produce a maximum effect. The effect of 1 mg. acetate increased with the age of the spores (table 11; fig. 3). Its action on young spores was more effectively supplemented with the D_R fraction than with hypoxanthine, but for spores 35 and 61 days old hypoxanthine was more effective than the D_R fraction. One mg. acetate affected the spores as would be expected if the Z factors were deficient in the young spores and increased as they aged. Germination on the agar containing 10 mg. acetate was maximum throughout.

TABLE 12

EFFECT OF AGE, ACETATE, AND Z FACTORS ON COLONY DIAMETERS (CF. TABLE 11)

Age of spores (days)	Diameter of colonies (mm.) after 28 hours on basal agar medium plus				
	Nothing	Hypoxanthine	D_R fraction	Hypoxanthine and D_R fraction	Potato extract
4	0	0	15	20	32
13	0	9	18	23	30
35	4	11	20	25	28
61	6	12	20	22	26
77	6	11	20	19	24
155	6	6	18	17	21
255	0	0	10	14	18
317	0	0	6	6	10
	Plus 1 mg. acetate				
4	6	10	22	22	.....
13	5	12	19	23	.....
35	10	16	22	24	.....
61	11	12	21	22	.....
77	7	13	19	20	.....
155	7	9	13	18	.....
255	5	5	12	13	.....
317	0	0	0	0	.....
	Plus 10 mg. acetate				
4	14	16	22	23	.....
13	12	15	20	23	.....
35	12	14	23	23	.....
61	11	14	17	22	.....
77	11	12	15	18	.....
155	8	9	14	15	.....
255	5	6	10	12	.....
317	0	0	3	4	.....

Although acetate had a marked effect on germination, its effect on colony growth was not so great as that of the Z factors. For example, the maximum growth in the basal agar plus 10 mg. acetate was 14 mm. (for the younger spores), while on the agar containing hypoxanthine and the D_R fraction it was 20 mm. for the same spores, and 25 mm. for spores 35 days old (table 12). In other words, although acetate markedly affected germination, its effect on growth was slight. This is evident (fig. 4) if the colonies produced from the spores 4, 13, and 35 days old on the agar containing 10 mg. acetate (9) are compared with those on the agar containing the two Z factors (4).

Discussion

It is assumed that the active factors concerned in breaking the dormancy of spores of *Phycomyces* and in improving its early mycelial growth in a basal mineral-dextrose medium containing thiamine and asparagine at 26° were hypoxanthine (factor Z_1) and factor Z_2, or physiologically equivalent substances. The action of other effective agents, including heat, low temperature, organic acids, pyridine, and soaking in water, is interpreted on the assumption that these treatments increased the quantity of Z factors available in the spores.

Although the final result seemed to be much the same, there was some difference in the conditions under which these various physical and chemical agents were effective. High temperature, pyridine, and soaking in water at 26° seemed

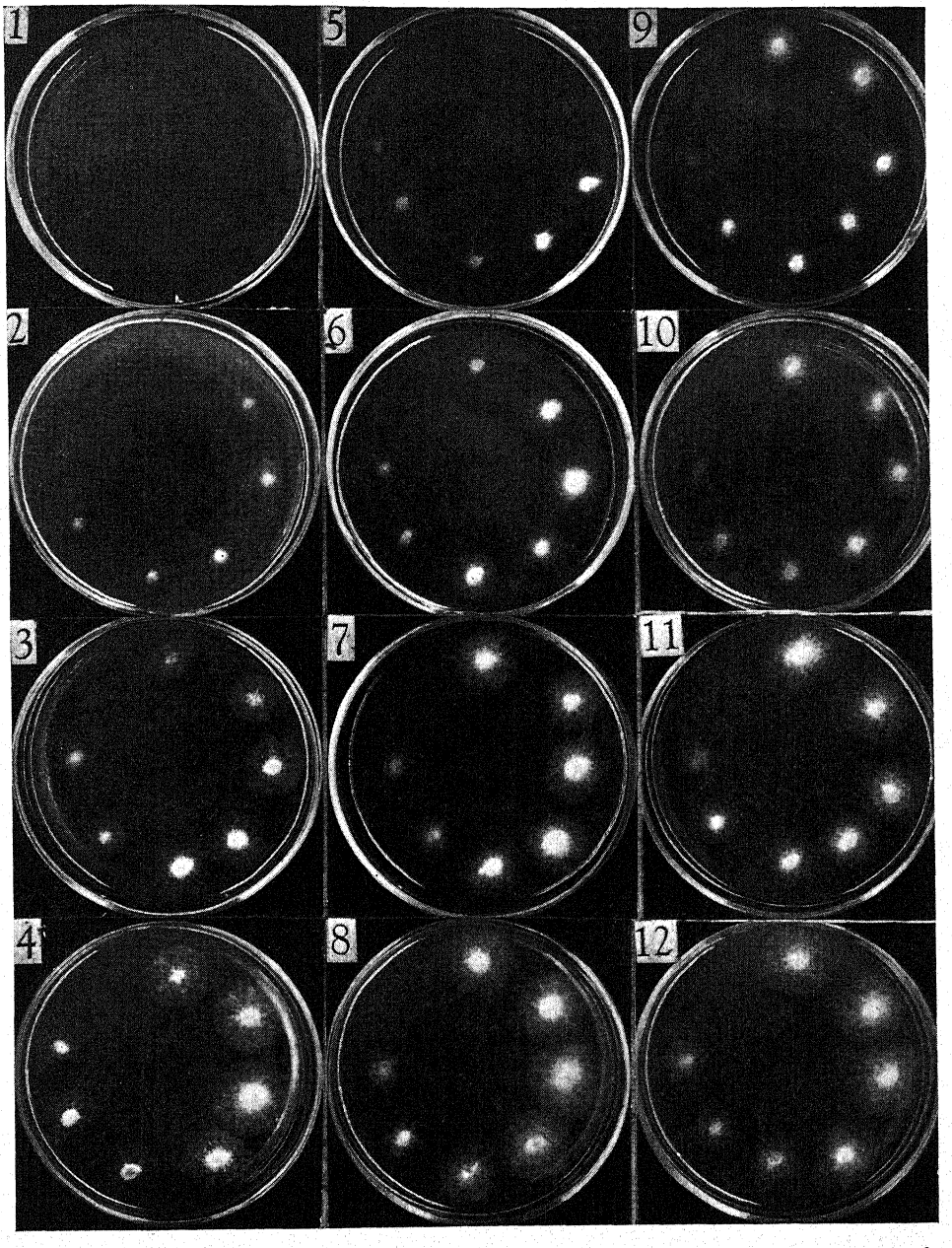


FIG. 4.—Colony growth of spores of *Phycomyces* of various ages on various media after 28 hours at 26° C. Age of spores in days on each plate beginning at top and proceeding clockwise: 4, 13, 35, 61, 77, 155, 255, and 317. Media as follows: *1*, *5*, *9*, basal medium; *2*, *6*, *10*, plus hypoxanthine; *3*, *7*, *11*, plus D_R fraction; *4*, *8*, *12*, plus hypoxanthine and D_R; *5*, *6*, *7*, *8*, plus 1 mg. acetate; *9*, *10*, *11*, *12*, plus 10 mg. acetate.

to set Z factors free, independent of the metabolic activity of the spore. On the other hand, low temperature and acetate appeared to act by inducing a type of metabolic activity in the spore which increased the quantity of available Z factors. Evidence for the validity of this hypothesis as an explanation for the effect of heat-activation may be summarized as follows:

Spores were demonstrated to contain material which affected germination and growth as the Z factors do.

The germination of partially heat-activated spores and the early mycelial growth of spores fully activated by heat agreed with the assumption that the quantity of these factors in the spores had been increased by the heating.

Material which affected early mycelial growth, as the Z factors do, diffused in greater quantity from heat-activated spores than from unactivated ones.

The evidence for a similar action by the other factors (acetate, pyridine, low temperature, and soaking in water) is not so complete. It is based chiefly on the fact that spores partially or completely activated by these agencies responded in media containing hypoxanthine or the D_R fraction as though the amount of the Z factors in them had been increased by the treatment concerned.

Although the hypothesis proposed is the most satisfactory devised so far, there are several questions in relation to it which need discussion.

Why are the Z factors not more specific in their effects on germination? A sufficient amount of either breaks dormancy. Several answers to this question may be suggested, none of which is entirely satisfactory.

The spore population may be mixed; that is, some spores contain sufficient available hypoxanthine but are not capable of germinating because of a lack of the Z_2 factor; others contain sufficient Z_2 factor but not enough hypoxanthine; a few contain enough of both and germinate on the basal medium.

There is also the possibility that there may be a kind of mass relation between these two factors, such that the product of their masses, within certain limits, induces germination and favors early mycelial growth.

A third possibility is that the D_R fraction, used here as a source of the Z_2 factor and which is a mixture of many substances, may be effective because it contains one of the active chemical agents; for example, organic acids. This interpretation is doubtful. The D_R fraction is active in amounts so small that if its activity were caused by any of the organic acids tested those acids would constitute 10 per cent or more of the fraction. This is not probable, as no migration of the active material to the anode chamber was observed when a D_R fraction was electrodialyzed. Furthermore, it has not been possible to duplicate the effect of the fraction on growth by any of the organic acids tested.

What evidence is there for assuming that the various chemical and physical agencies act through the intermediary of the Z factors and not direct? The most important evidence is that none of the treatments (heat, acetate, pyridine, etc.) was as effective as the addition of hypoxanthine and factor Z_2 (in the D_R fraction) to the medium. This was not true for germination but applied to early mycelial growth. For example, although acetate produced maximum germination it had little effect upon growth and did not replace the D_R fraction in this respect. The growth of heat-activated spores was

improved by the addition of the Z factors to the medium. Similar considerations may be applied to the other treatments. If one of the other factors was the primary agent, then it should be possible to duplicate by its use the effects of the Z factors. On the other hand, if the other factors act through the Z factors as intermediaries, spore treatments would be expected to produce less than enough to give maximum effects on later growth. This would be because the quantity of Z factors released in a spore as the result of any specific treatment must of necessity be minute and insufficient to produce maximum effect on later growth, even though the quantity was adequate to induce germination.

Of other explanations which might be suggested, change in the permeability of the spore membranes seems most probable. It might be assumed that the Z factors, heat, low temperature, organic acids, and pyridine affect germination by increasing the permeability of the membranes. However, this would not account for effects noted on later growth.

In fact, the hypothesis which assumes the Z factors as the primary agents concerned appears to fit the results better than any other. A final decision on its validity will probably not be possible until the Z_2 factor has been identified. Discussion of the application to other examples of dormancy of these results and their explanation would not be profitable at this time. The similarity between the treatments found effective in breaking the dormancy of spores of *Phycomyces* and those used for other dormant organs or entire plants is obvious. It seems reasonable to assume that dormancy in some instances results from a temporary deficiency of one or more essential metabolites. Time or a suitable treatment supplies or induces the formation of the deficient and essential substances, and breaks or shortens the dormant period.

Summary

Spores of *Phycomyces* germinate poorly at 26° C. on a mineral-dextrose agar medium containing thiamine. The percentage of germination increases somewhat with age of the spores, reaching a maximum of about 12 per cent after $2\frac{1}{2}$ months. It is affected slightly by the temperature at which the spores are grown and by their exposure to light during development. The addition to the medium of extracts of potato tubers or other natural products, of hypoxanthine and a D_R fraction (Z factors), or of acetate and some other organic acids increases germination to nearly 100 per cent. Treatment of spores with aqueous pyridine, 2 hours' exposure to temperatures of 11° or 15°, or treatment for 30 minutes to 1 hour at 50°, has the same favorable effect. Failure of the spores to germinate on the basal medium is interpreted as a dormancy phenomenon. The dormant spores are considered to lack sufficient available Z factors for germination. The extracts of natural products or the Z factors furnished in the medium supply the deficiency, which may also be met by treatment with heat, cold, acetate, or pyridine. These treatments are thought to change the Z factors in the spores from an unavailable to an available form.

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LITERATURE CITED

1. AVERY, O. T., and MORGAN, H. J., Studies on bacterial nutrition. V. The effect of plant tissue upon the growth of anaerobic bacilli. Jour. Exp. Med. **39**:289–302. 1924.
2. ROBBINS, W. J., Growth substances and gametic reproduction by *Phycomyces*. BOT. GAZ. **101**: 428–449. 1939.
3. ———, Growth substances in agar. Amer. Jour. Bot. **26**:772–778. 1939.
4. ———, Effect of extracts of *Phycomyces* upon its development. Amer. Jour. Bot. **27**:559–564. 1940.
5. ———, Further observations on factor Z. BOT. GAZ. **102**:520–535. 1941.
6. ROBBINS, W. J., and HAMNER, K. C., Effect of potato extracts on growth of *Phycomyces*. BOT. GAZ. **101**:912–927. 1940.
7. ROBBINS, W. J., and KAVANAGH, F., Guanine and factor Z_1, growth substances for *Phycomyces*. Proc. Nat. Acad. Sci. **28**:4–8. 1942.
8. ———, and ———, Hypoxanthine, a growth substance for *Phycomyces*. Proc. Nat. Acad. Sci. **28**:65–69. 1942.
9. ROBBINS, W. J., and KAVANAGH, VIRGENE, Vitamin deficiencies of the filamentous fungi. Bot. Review **8**:411–471. 1942.
10. MORGAN, H. J., and AVERY, O. T., Studies on bacterial nutrition. IV. Effect of plant tissue upon growth of *Pneumococcus* and *Streptococcus*. Jour. Exp. Med. **38**:207–217. 1923.

HISTOLOGICAL RESPONSES OF VICIA FABA TO INDOLEACETIC ACID

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 548

BARBARA F. PALSER

(WITH TWENTY-THREE FIGURES)

Introduction

The gross and histological responses of several varieties of plants to various growth-regulating substances, applied laterally to intact and apically to decapitated stems, have been reported. A few recent papers (8, 10, 19) suggest that certain growth substances may induce polyploidy in nuclei of the treated organs. There is no evidence recorded for bean (17), *Iresine* (14), tomato (6), or *Lilium* (1) that indoleacetic acid does induce polyploidy. On the contrary, BEAL found evidence in *Lilium* indicating that all stages of mitosis were regular and chromosome structure normal throughout division.

For the present investigation, plants of *Vicia faba* were grown in pots containing garden soil under ordinary greenhouse conditions during the autumn, winter, and spring of 1941–1942. Following germination, the cotyledons and the first or cotyledonary node of *Vicia* remain underground; small bracts are present at the second and third nodes, and the first true leaf with its large stipules is at the fourth node. Stems were decapitated at the upper end of the fourth internode, just below the fifth node, at the time the second true leaf was beginning to expand and the fifth internode beginning to elongate slightly (fig. 1*A*). The cut surface was covered with a layer of a 1.5 per cent mixture of indoleacetic acid in lanolin or with lanolin alone.

The tips of the treated plants were collected at hourly intervals from 15 to 48 hours following treatment, and after that at 4-hour intervals up to 104 hours. Others were allowed to grow for a month or more before collection. The tips were killed and fixed in Navashin's solution, and imbedded according to the butyl alcohol-paraffin method. Sections were cut at 10 μ and stained in a modified triple stain.

Plants of a series grown in late spring and early summer, 1942, and treated as before, were collected at intervals, sectioned freehand, and stained with iodine-potassium iodide solution. These sections showed the effects of treatment on starch stored in the endodermis and on the chloroplasts present in the outer cortex.

Observations

GROSS RESPONSES

During the first 24 hours after treatment little or no change could be observed. Occasionally, if the tissue when treated was in a younger, more immature stage than usual, a very slight swelling could be seen. During the next 24 hours the swelling of the tip, although never very great, became distinct. The bright green of the stem became paler in the swollen region. The amount of swelling increases with length of time after treatment (fig. 1*C–F*). The plants treated with lanolin alone showed no similar

swelling or decrease in color (fig. 1*B*), and on them lateral shoots developed rapidly at the second and third nodes. Shoots also developed on plants treated with indoleacetic acid but were slower in starting and somewhat slower in later development. The tumors that had developed for a month or more showed a slightly darkened, roughened surface, and some showed a small amount of callus proliferation. Plants decapitated and left untreated showed a dry exposed surface but otherwise little change over a considerable period of time. The relative size of tumors formed on stems of *V. faba* following treatment was much smaller than those formed on bean in a corresponding length of time. A young tumor may extend 2–3 mm. back from the cut surface and the fading of color slightly farther. Old tumors do not extend much farther back in the stems than young ones, the longest extending 3–5 mm. The increase in diameter varies from an amount barely visible to once and a half or twice the original diameter.

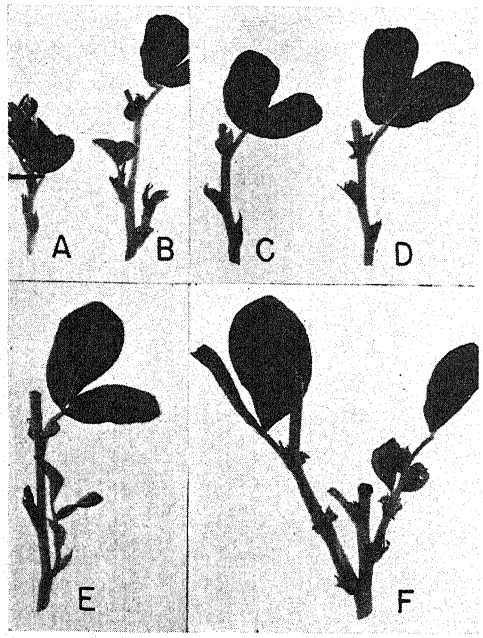
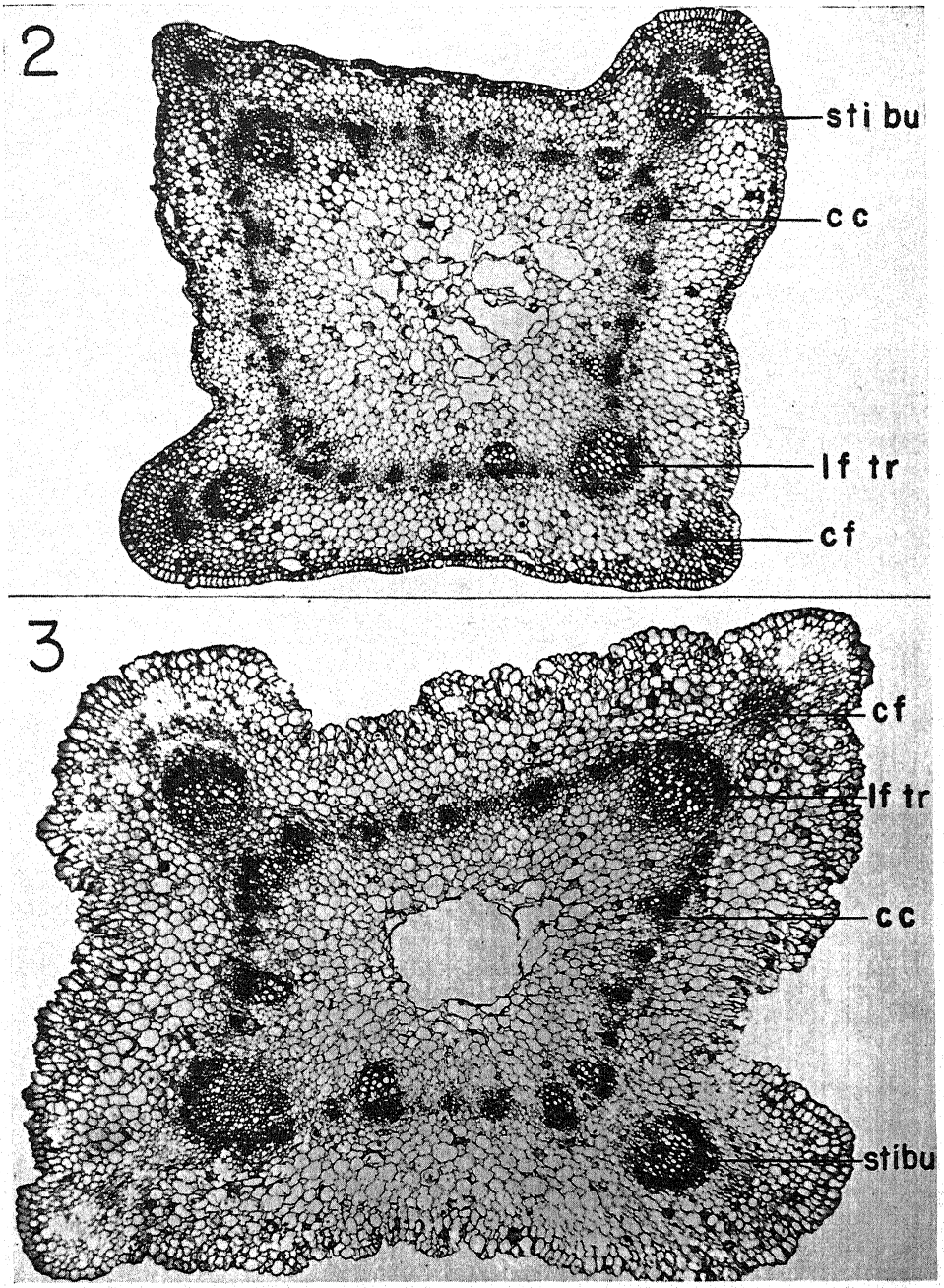


FIG. 1.—*A*, *V. faba* at time of treatment, showing region of decapitation. *B*, decapitated plant treated with lanolin alone, 94 hours after treatment. *C–F*, decapitated plants treated with 1.5 per cent indoleacetic acid in lanolin (*C*, 30 hours after treatment; *D*, 71 hours; *E*, 94 hours; *F*, 30 days after treatment).

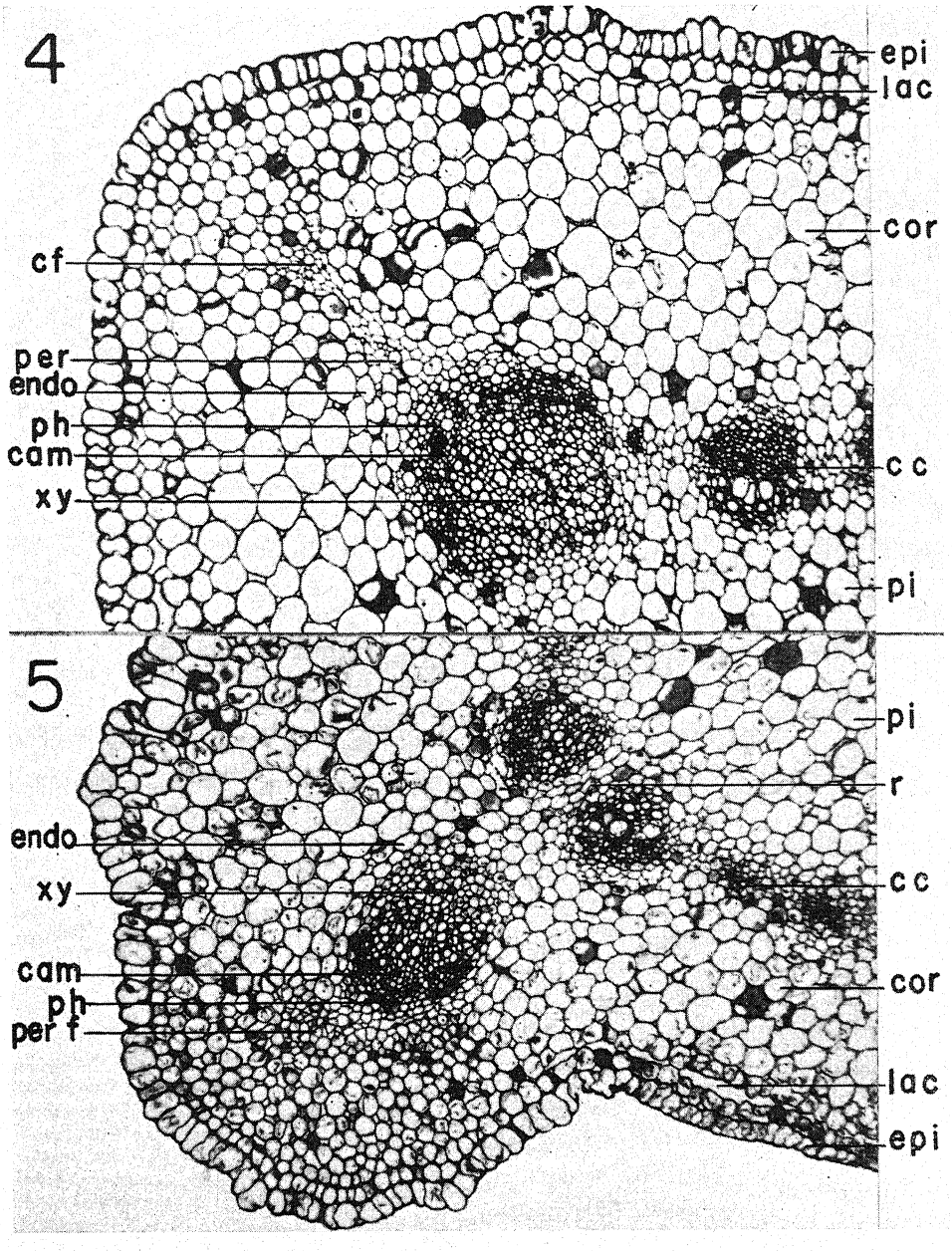
HISTOLOGICAL DETAILS

The transition region in *V. faba* extends through the first two internodes and is in many respects similar to that in pea (**7, 24, 15**). Typical stem anatomy is present in the treated fourth internode. At the time of treatment many of the cells are still immature, but the regions are differentiated and can be easily distinguished. Figure 2 shows a cross-section of the fourth internode 32 hours after treatment with lanolin alone. A central cylinder of vascular bundles is evident, with two polar bundles much more prominent than the others, the leaf traces for the leaves at the fifth and sixth nodes. In the cortex outside each trace is a small bundle of fibers which departs into the leaf with the leaf trace. Such a leaf trace and cortical bundle are shown in figure 4. The leaf gap is closed by the adjacent bundles of the central cylinder. Large fibrovascular bundles are also present in the cortex in the corners alternate with those in which the leaf traces are situated. These are the stipular bundles (fig. 5). At the fifth node each stipular bundle divides into two, one-half supplying the stipule and the other half remaining in the cortex through the fifth internode. The bundles of the central cylinder vary in size and are separated by parenchymatous rays of differing widths. Rays one cell in width are formed in the secondary xylem.

Surrounding the vascular cylinder and the stipular bundles is an endodermis,



FIGS. 2, 3.—Cross-sections of fourth internode of *V. faba* just below fifth node. Fig. 2, 0.85 mm. from cut surface; 32 hours after treatment with lanolin alone. Fig. 3, 0.85 mm. from surface; 31 hours after treatment with 1.5 per cent indoleacetic acid in lanolin. *sti bu*, stipular bundle; *lf tr*, leaf trace; *cf*, cortical strand of fibers; *cc*, central cylinder.



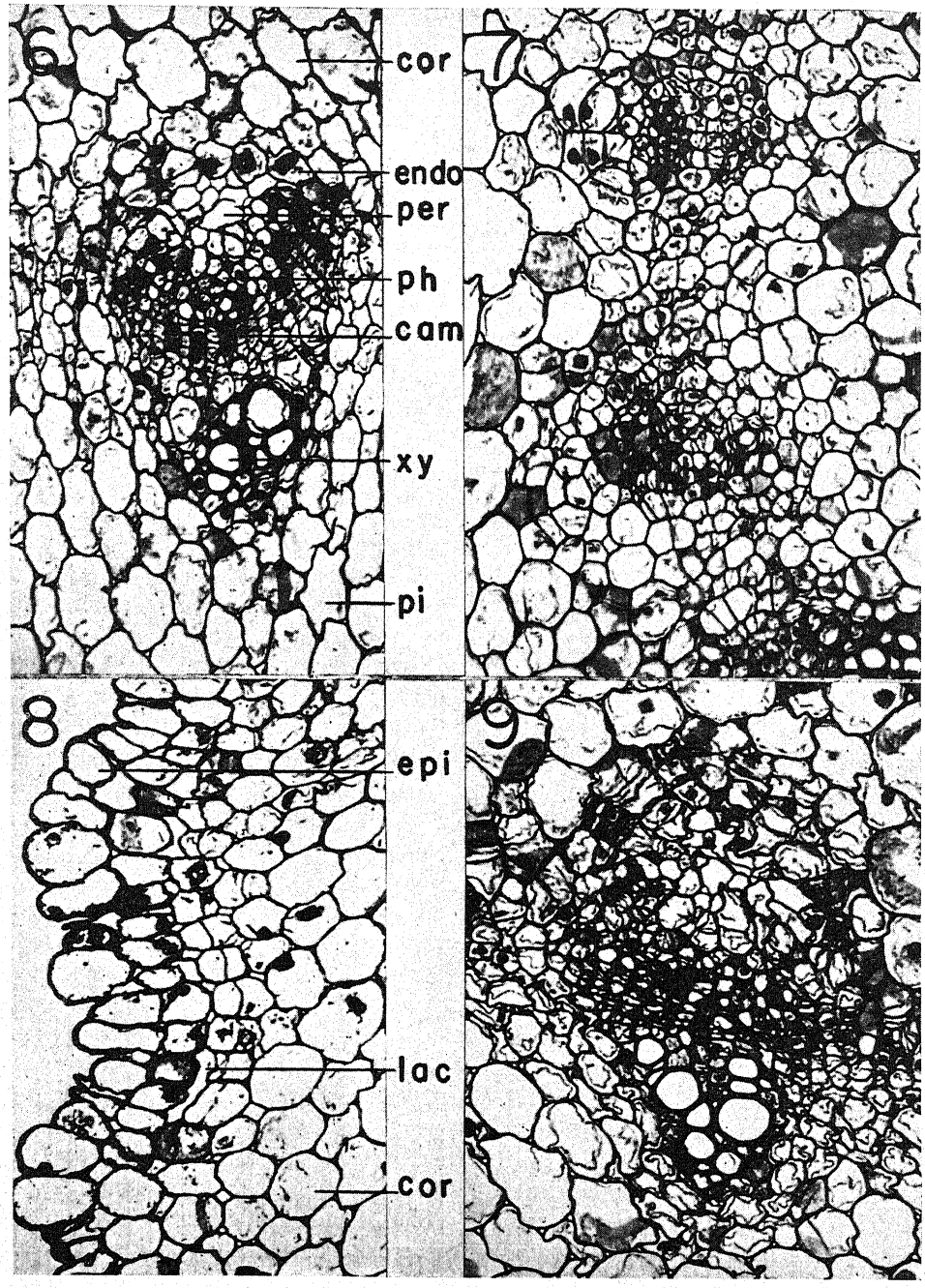
FIGS. 4, 5.—Fig. 4, 0.6 mm. below surface; portion showing leaf trace and cortical strand of fibers 96 hours after treatment with lanolin alone. Fig. 5, 0.9 mm. from surface; portion showing cortical stipular bundle 56 hours after treatment with lanolin alone. *epi*, epidermis; *lac*, lacuna; *cor*, cortex; *pi*, pith; *per*, pericycle; *per f*, pericyclic fibers; *endo*, endodermis; *ph*, phloem; *cam*, cambium; *xy*, xylem; *r*, ray.

the cells of which are not clearly differentiated but which is easily recognizable in fresh sections stained with iodine because of the presence of stored starch. Associated with each larger bundle is a small group of pericyclic fibers, largely immature, often separated from the phloem by a few parenchymatous cells. A fascicular cambium is usually present in the larger bundles but not always in the smaller ones. With increased age a fascicular cambium develops in the smaller bundles, and an interfascicular cambium, which may give rise to a few derivatives, forms between the bundles. The cortex consists of parenchymatous cells, rounded in cross-section, with intercellular spaces. The outer four or five layers of cells contain chloroplasts. In the outer part of the cortex between the first and second layers under the epidermis small lacunae are present. The epidermis is made up of rather small cells, rounded or radially rectangular in cross-section, and is covered with a thin layer of cutin. It contains numerous stomata, and there are a few glandular hairs. The cells of the pith are parenchymatous and rather large. Usually there is complete disintegration of the innermost ones, leaving the stem hollow.

The initial changes following treatment with indoleacetic acid are so gradual that it is difficult to determine when they first begin. By 30 hours after treatment, however, certain characteristic differences can be seen (fig. 3). The epidermal cells and outer layers of the cortex have enlarged in radial dimension. Chloroplasts have almost entirely disappeared from the enlarged cells. The enlargement causes the margin of the stem, which in an untreated plant or in one treated with lanolin alone is regular in outline, to be thrown into convolutions. At the same time the cells of the cambium have become somewhat more active. The region of undifferentiated derivatives is slightly wider, and the nuclei appear larger. In the parenchymatous cells of the cortex, rays, and pith, activity appears to be somewhat increased, as some of the nuclei are larger and there are more division figures observable than in a stem treated with lanolin alone for the same number of hours.

Development subsequent to this beginning is shown in figures 6–23. Although plants were collected at frequent intervals following the appearance of early differences, only those were photographed which showed a definite increase in response. All plants did not respond at exactly the same rate. Differences can be associated with the amount of lanolin mixture spread on the surface, with the maturity of the tissues at the time of treatment, and probably also with growing conditions at that time. Changes were initiated sooner and development proceeded more rapidly in those plants that were highly vegetative at the time of decapitation.

The changes observable at 30 hours increase with length of time after treatment, both in amount of response in regions already referred to and in number of regions responding. At 36 hours some of the endodermal cells have increased slightly in size, but more striking than this is the increase in size of the nuclei and the initiation of their division (fig. 6). An occasional endodermal cell divides in a tangential plane. In the region where this activity begins, just below the cut surface, starch has disappeared but is still present farther away from the surface, where activity in the endodermis has not yet begun. Early divisions of the endodermis always appear in connection with a vascular bundle, either large or



FIGS. 6–9.—Fig. 6, 0.95 mm. from surface; 36 hours after treatment, showing enlarged nuclei of first division of endodermis outside a bundle. Fig. 7, 1.05 mm. below surface; 52 hours after treatment, showing divisions in endodermis, formation of interfascicular cambium, and increase of cytoplasm. Fig. 8, 0.4 mm. from surface; 38 hours after treatment, showing tangential divisions in outer cortex. Fig. 9, 0.8 mm. below surface; 92 hours after treatment, showing repeated divisions in two planes within one endodermal cell forming provascular strand.

small. After 40 hours one division and occasionally a second have occurred in cells around the bundles; after 48 hours almost all the endodermal cells over bundles have begun to divide (fig. 7); after 72 hours the greater part of the derived endodermal layer is three or four cells thick; while at 80 hours divisions are beginning in the endodermal cells between the bundles, forming an almost continuous region of proliferated tissue around the stem. The extensions of the endodermis around the leaf traces, whether including the cortical fiber bundle or not, are evident (fig. 10). Between 84 and 92 hours the derivatives of one, sometimes two, endodermal cells undergo a series of divisions in various planes without subsequent enlargement, forming a compact group of small, very active cells (fig. 9). They appear to be provascular strands. The endodermis and cortex around the cortical fiber bundle divide, at first tangentially and later in several planes, forming provascular strands.

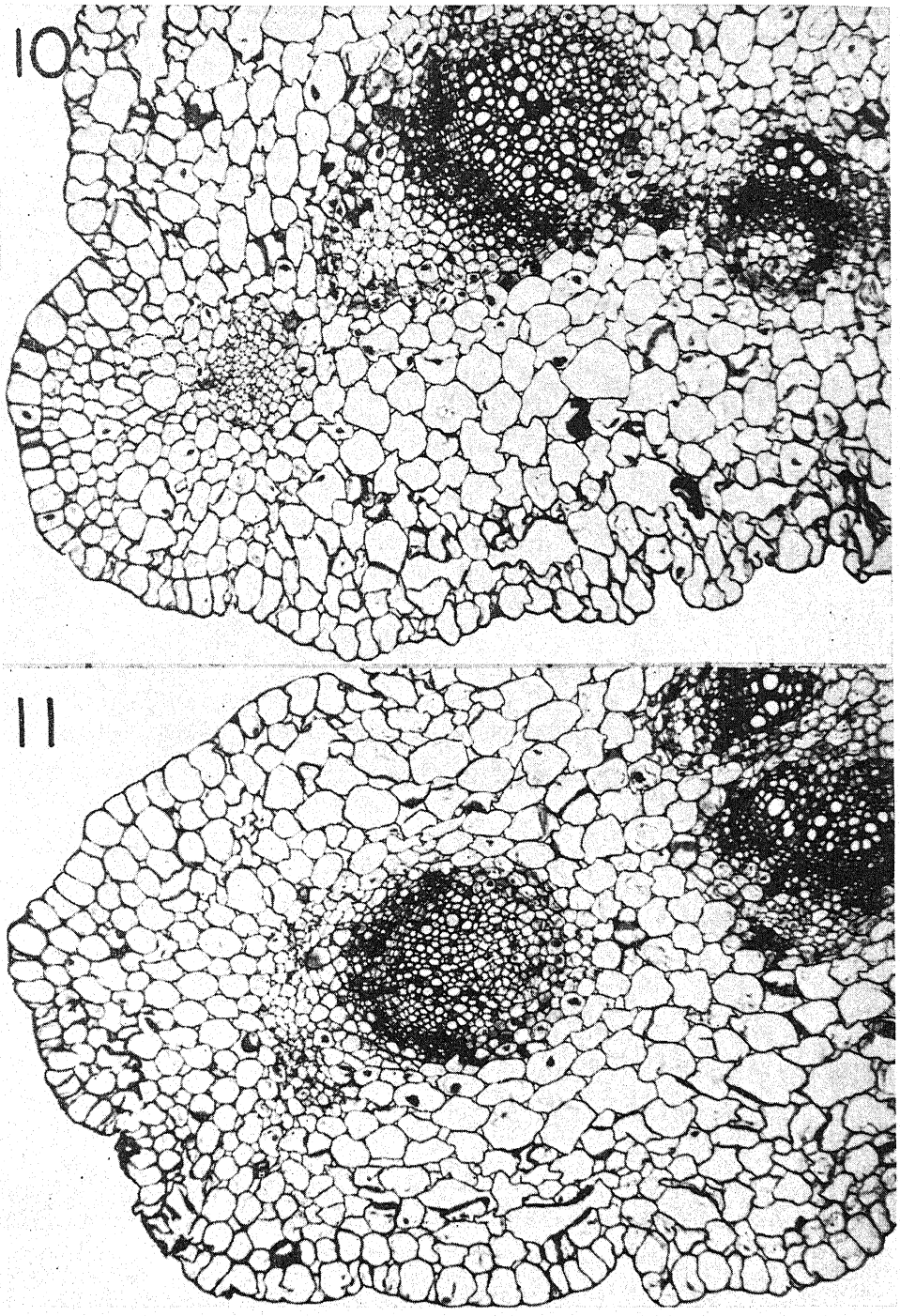
In a longitudinal section of a tumor 72 hours old the endodermis can be followed from a layer one cell wide at the surface to a layer three, four, or five cells wide immediately below the surface, and then narrowing again below, but more gradually, to a single layer. Portions of the endodermal cells sometimes appear to project into the pericyclic fibers next to them, similar to the projections of parenchymatous cells into vessels forming tyloses. Nuclei migrate into the projections and there undergo division.

After 40 hours, in some places in a few stems tangential divisions can be seen in the second cell layer of cortical parenchyma under the epidermis (fig. 8). This type of division does not appear at all consistently but is found occasionally in tumors of varying ages. It may perhaps be associated with the presence of a little lanolin mixture on the side of the stem as well as on the surface. In spite of care to put the mixture only on the decapitated surface, occasionally a little may get on to the side.

The amount of cytoplasm in the active cells tends to increase gradually after 36 hours but never becomes as dense as it does in bean. Activity in the cambium increases with length of time after treatment, older stems showing a noticeable development of secondary xylem. At 40 hours there are indications of the beginning of an interfascicular cambium, which become more evident after 48 hours (fig. 7). At 72 hours it is rather well developed, and at 80 hours its activity has almost completed the vascular cylinder (fig. 10).

By 48 hours after treatment cells scattered through cortex and pith show nuclei in various stages of division, together with cells which appear to have recently divided. These scattered divisions are chiefly transverse as seen in longitudinal section. Transverse divisions would account for the rather large number of mitotic nuclei observed in cross-sections, in contrast to the relatively small number of recently divided cells observed in cross-sections of even older tumors (up to 100 hours). They would also account for the increase in number of nuclei visible in cross-section of a treated stem, as the resulting cells are shorter in longitudinal dimension.

Parenchymatous cells of the phloem may show slight activity after 48 hours. Ray cells sometimes divide, particularly transversely, in the early stages. A longitudinal section may show some cells with two or three nuclei and no cross walls, but this is rather infrequent. Cambium, xylem, phloem, and ray parenchyma are active in early callus proliferation when callus is formed. By 72 hours in those tu-



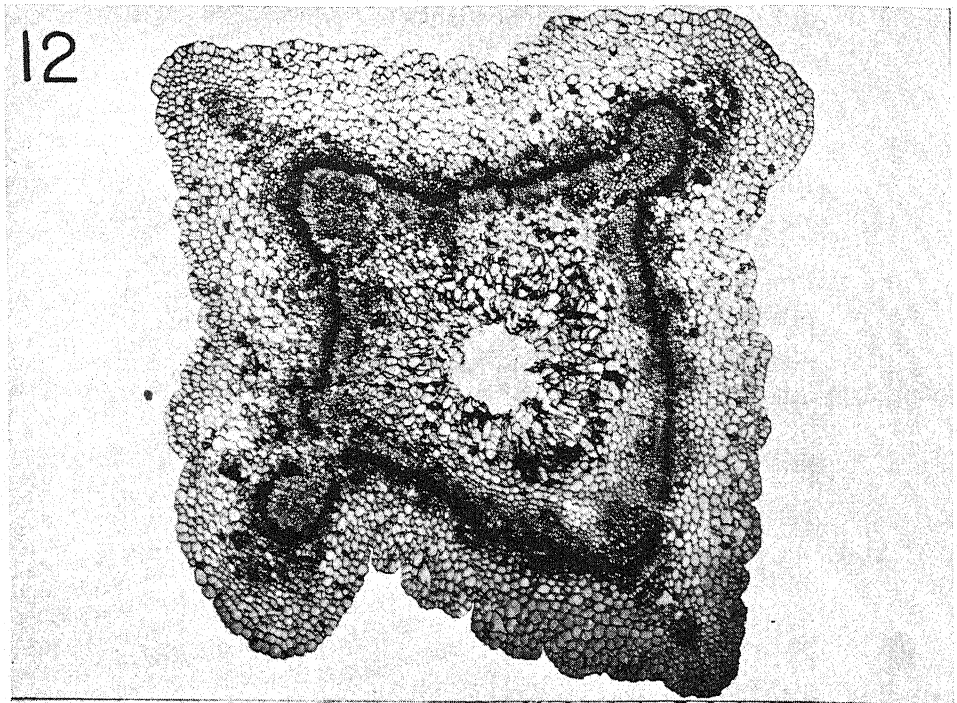
FIGS. 10, 11.—Seventy-two hours after treatment. Fig. 10, 1.1 mm. below surface; activity in endodermis around leaf trace; cortical strand of fibers outside central cylinder. Fig. 11, 1.6 mm. below surface; stipular bundle becoming active at inner edge.

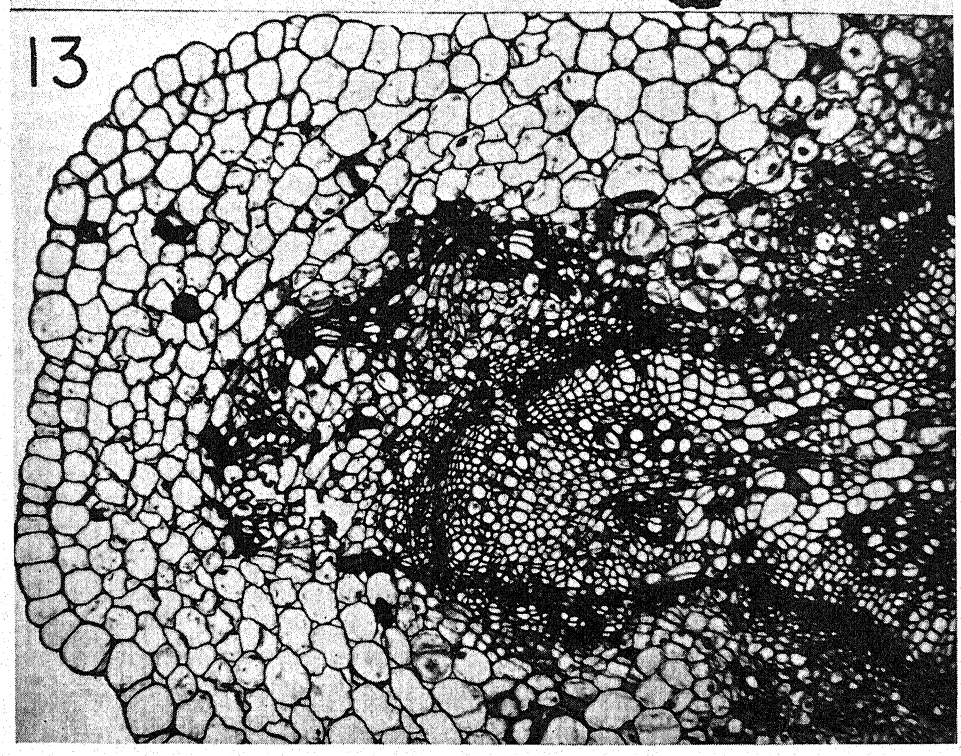
mors which do form callus the living cells just below the cut surface have begun to elongate upward and to divide, beginning the formation of a callus over the surface. In much older tumors other parenchymatous cells also become active and add to the proliferation.

By 72 hours parenchymatous cells at the lateral and inner edges of the stipular bundles have become active and show several derivatives (fig. 11). Later such cells between the stipular bundle and the central cylinder may divide. None of the active regions extends more than 2–3 mm. back from the cut surface. The activity in the fascicular and interfascicular cambium, the enlargement of the cortical and epidermal cells, and occasionally the divisions of the endodermis appear farthest from the surface. The largest part of the tumor up to 100 hours after treatment is due to the radial enlargement of the cortex and epidermis.

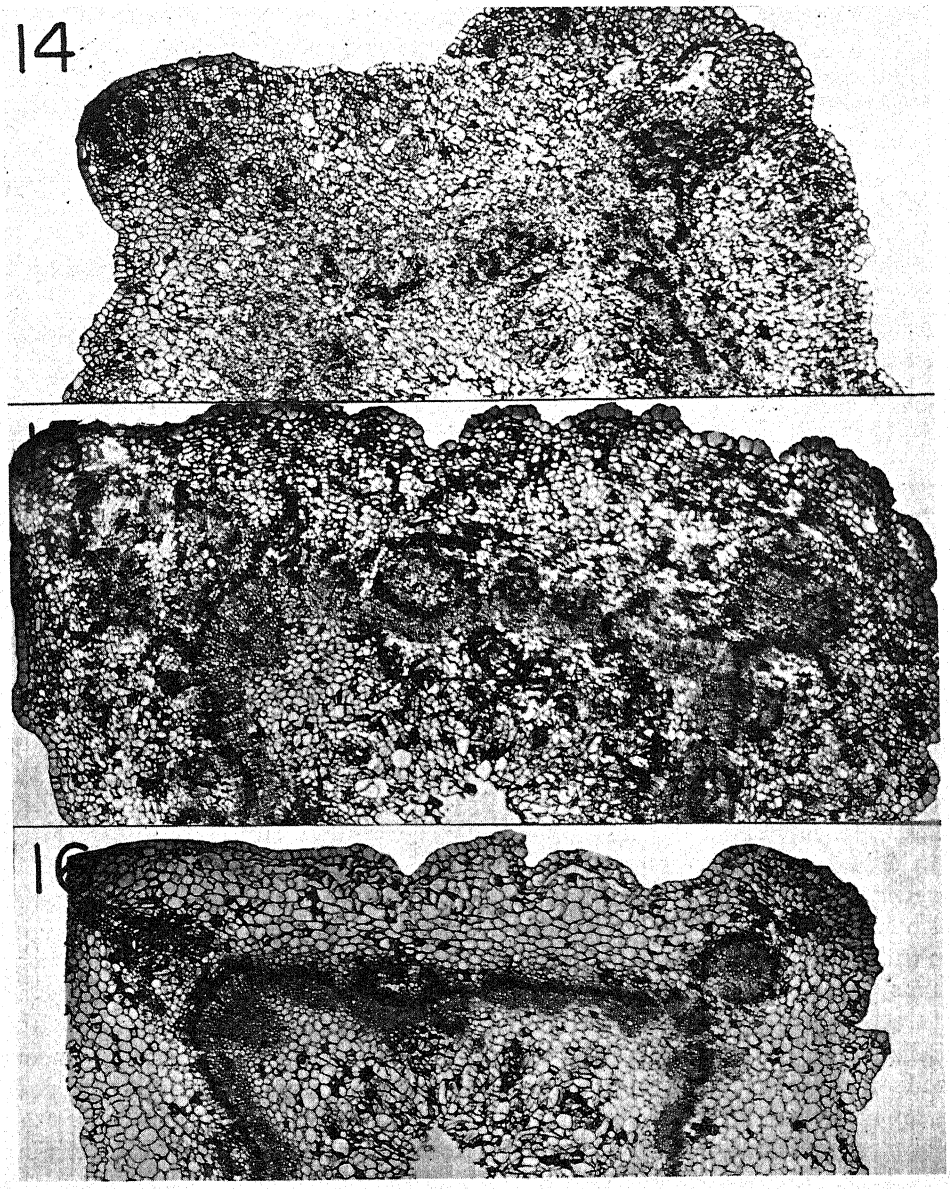
Development of tumors subsequent to the first 100 hours has not yet been followed closely, but later responses will be described briefly from several older tumors of widely separated ages. After 7 days, responses already described have increased. Owing to the continued activity of the fascicular and interfascicular cambium, the vascular cylinder is almost complete around the stem, with a great deal of secondary xylem (fig. 12). Activity of the cells around the stipular bundles, especially laterally, has resulted in a continuation of the cambium, with xylem differentiating on the inner side and phloem on the outer. This development leads to a bundle, horseshoe-shaped in outline, with secondary xylem completely surrounding the primary strand except for a small area nearest the stele (figs. 12, 13). The endodermis has continued activity and in many places shows small vascular strands starting to differentiate. In some places the cortical cells next to the endodermis show an increase in both cytoplasm and divisions (fig. 13). Some of the pericyclic fibers, evidently immature at time of treatment, and some pericyclic parenchymatous cells, have become active and may show several derivatives. The walls of others of the fibers have thickened and remain single, or in small groups, scattered among the active cells. In one or two instances there are a few cells in the cortex, grouped together, which have divided repeatedly to form provascular masses. At this time, however, the largest part of the cortex is unresponsive to the treatment. The pith, which earlier showed little response, has become highly active. The parenchymatous cells bordering the hollow center have proliferated inward, forming a callus in the hollow portion. The cells of the callus and those next to it have divided repeatedly, in many places tangentially and in others in various directions, to form provascular masses.

Tumors a month or more old show a still greater response. A short distance below the cut surface is found the region of greatest activity (fig 15). The surface of application may be covered with a thin layer of callus, in which wound tracheids may have differentiated irregularly (fig. 14). In the active region the proliferated endodermis has given rise to vascular strands. Some cells of the pericycle are active in central cylinder bundles and in stipular bundles. In connection with the leaf traces, cells of the primary phloem have become active, forming vascular strands to increase the amount of extra-vascular tissue already differentiated in this region from pericycle and endodermis. The strands derived from endodermis, pericycle, and phloem are distinct from one another but





FIGS. 12, 13.—Seven days after treatment. Fig. 12, 0.65 mm. below surface; cross-section showing continuous vascular cylinder, large amount of secondary xylem, callus proliferation inward from pith cells, activity in endodermis, beginning activity in cortex (lower left corner). Fig. 13, 0.5 mm. from surface; same stipular bundle as in upper right in fig. 12 enlarged. Bundle does not usually become continuous with central cylinder as shown here. Note vascularization in endodermis, activity in pericycle, activity of inner cortex.



FIGS. 14–16.—Cross-sections at different levels of 35-day-old tumor. Fig. 14, 0.25 mm. from surface; largely callus. Fig. 15, 0.85 mm. from surface; activity of cortex, pith, and vascularization of endodermis. Fig. 16, 2.5 mm. below surface; evidences of activity at this level in pith, cambium, endodermis, and around stipular bundle.

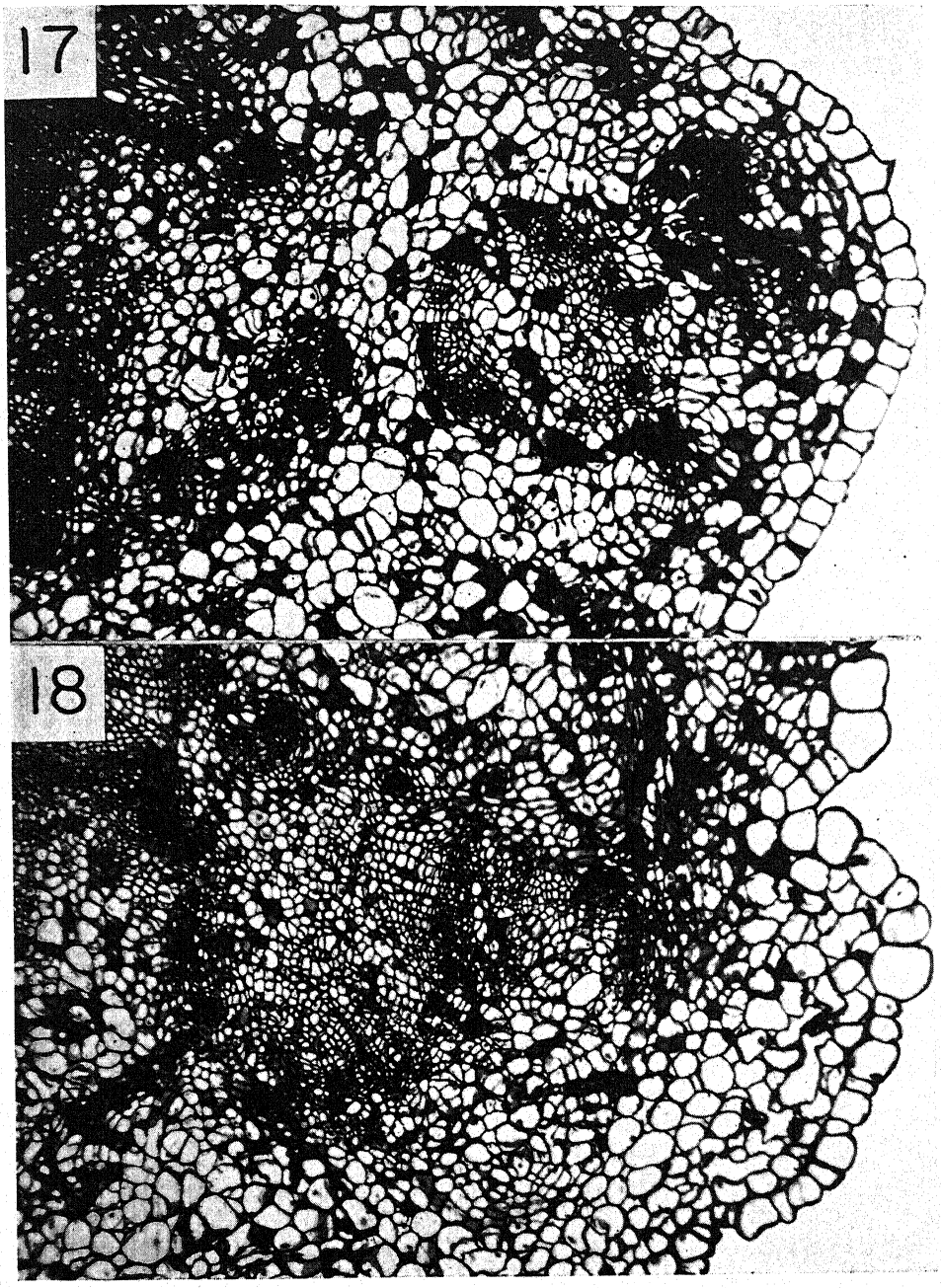
form a zone around the central vascular cylinder (figs. 15, 19, 20), around the stipular bundles joining them to the central cylinder (fig. 18), and around the large zone of pericycle outside the leaf traces (fig. 17). Many of the extra-vascular strands differentiate a cambium which is active in forming xylem toward the center of the stelar region and phloem to the outside. When the endodermis was continuous around the group of pericyclic fibers in the cortex, the extra-vascular strands derived from it are also continuous around the bundle. Strands found between the phloem and pericycle, evidently formed from the phloem, also develop a cambium, but differentiation of its derivatives is the reverse of that in the endodermal strands. Xylem is formed toward the pericyclic mass and phloem toward the phloem of the leaf trace. There is thus formed a complete new vascular ring, largely outside the main vascular cylinder (note leaf trace in the upper lefthand corner of fig. 15). Where the groups of fibers are separate from the leaf trace, the extra-vascular strands are between the cortical group and the leaf trace. The group of fibers, however, is surrounded by endodermis, which has formed—probably in conjunction with cortical parenchyma—vascular strands of the same type as around the leaf trace proper (fig. 17).

Cells of the cortex, both next to the endodermis and just under the epidermis, are active and may show as many as ten or more small cells within one mother cell wall. Vascular masses also differentiate in the cortex. Pith cells become active right up to the vascular bundles and may form vascular strands or masses of tracheids near the xylem of the main vascular cylinder (fig. 19). Callus in the hollow portion of the pith is extensive. Between 0.5 and 1 mm. from the treated surface hardly a single cortical or pith cell remains inactive, and small vascular and meristematic masses are numerous (fig. 15). Occasionally tangential divisions in the epidermis may be found.

Vascular strands in the endodermis (particularly in association with the leaf traces), the horseshoe development of the stipular bundles, the formation of callus from the pith cells, the activity of fascicular and interfascicular cambium with differentiation of secondary xylem, and the enlargement of cortical and epidermal cells—all extend farthest from the cut surface in the old tumors (figs. 16, 20). Division figures are scarce in old tumors. Apparently continued active proliferation has stopped.

In all the tumors cut, only three showed evidences of root formation. In only one were true root primordia located. This tumor had two primordia occurring at distances of 2 and 3 mm. below the cut surface. They show differentiation into epidermis, cortex, and stele and have a protective cap over the tip, but the histogenic region is not clear. They extend a short distance into the cortex from the vascular region (fig. 21). As these are the only organized roots found and no initial stages have been observed, it is extremely difficult to be sure from what tissue they are derived. As far as can be ascertained, they are formed in the region of a ray of the central cylinder. The endodermis may be involved in part in the formation, but probably the greater portion is derived from cells inward from it.

In addition to these organized root primordia, several peculiar structures were observed. In one tumor 7 days old one small and two large masses of proliferated tissue appear a short distance back from the treated surface. The masses consist of small cells with a great



FIGS. 17, 18.—Thirty-five days after treatment. Fig. 17, 0.85 mm. from surface; leaf trace showing separate vascularization of endodermis around cortical group of fibers. Note activity and groups of wound tracheids in cortex. From same section as fig. 15 from portion not shown in fig. 15. Fig. 18, 0.85 mm. from surface; enlargement of upper right corner of fig. 15; stipular bundle showing extra-vascular bundles joining bundle to central cylinder. Note activity of cortex.

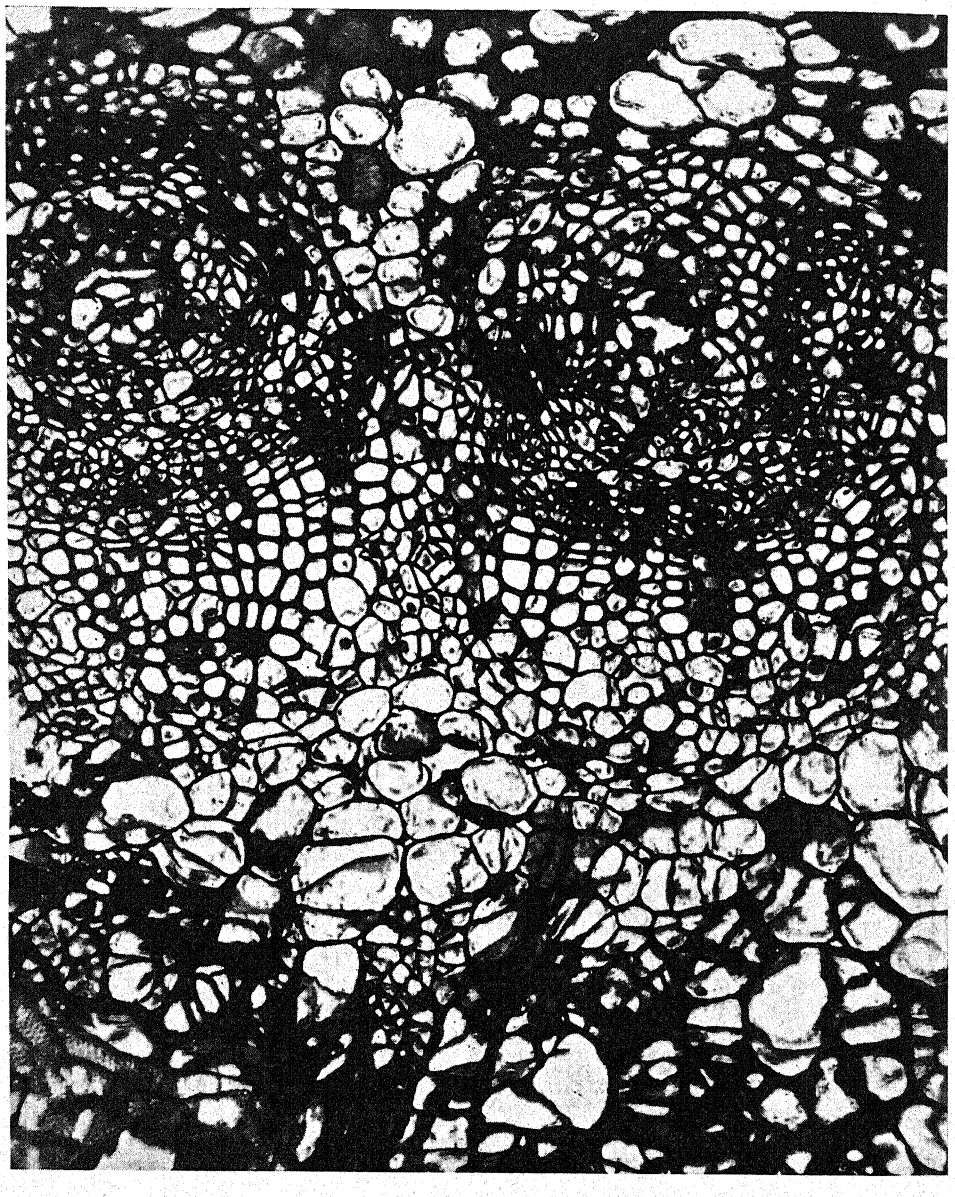


FIG. 19.—Thirty-five days after treatment, 0.85 mm. below surface; enlargement of two small bundles shown in fig. 15. Small bundles of central cylinder showing extra-vascular strands of endodermis, activity of phloem, ray, and pith; thickenings on wound tracheids in pith.

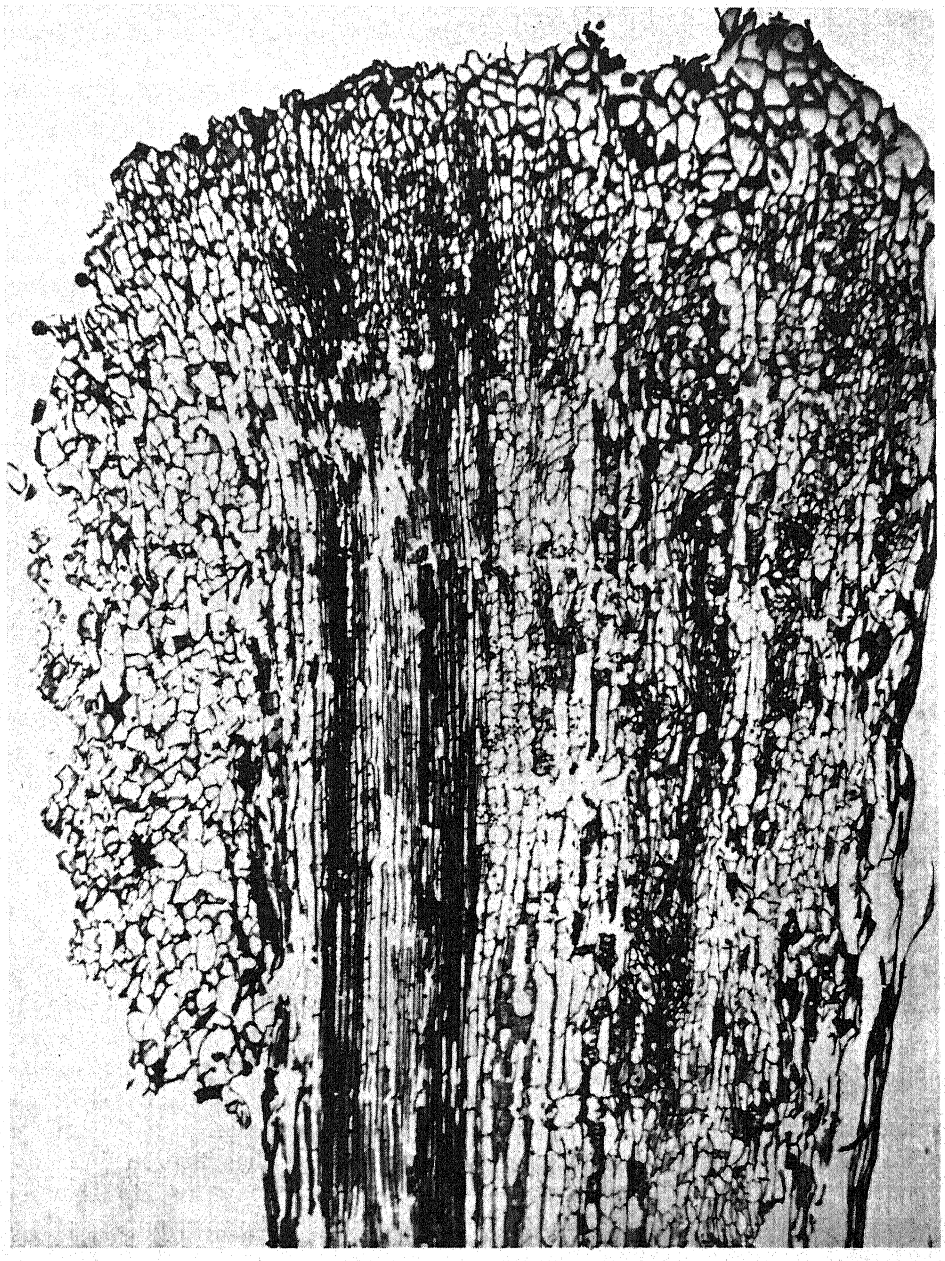


FIG. 20.—Diagonal section of 35-day-old tumor through leaf trace, showing strand in endodermal region, proliferation in cortex, activity of phloem and pericycle, and both pith and surface callus.

deal of cytoplasm and large nuclei. The cells are in active process of division. The group of cells shows no organization of any kind. It has not been possible to determine their derivation accurately, but the endodermis appears to have been



FIG. 21.—Longitudinal section of root primordium 2 mm. from surface of 46-day-old tumor.

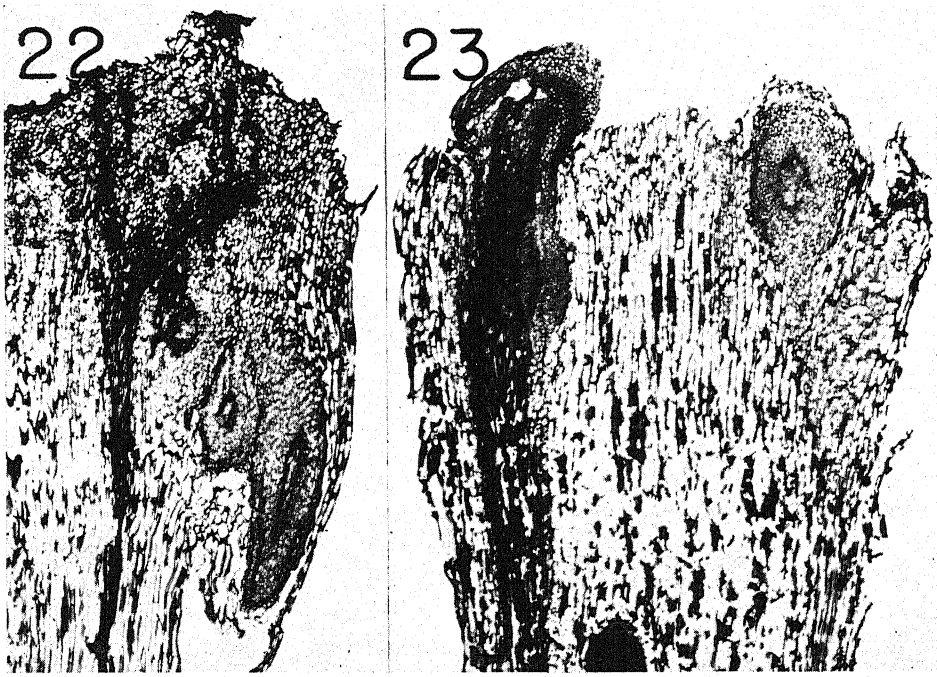
particularly active. In two older tumors proliferations having no particular organization were found. They show differentiated tracheids in the region adjacent to and in contact with the vascular strand of the stem; the differentiated tracheids are continued by regions of smaller cells almost to the edge of the proliferation. The cells are not meristematic; their cytoplasm is highly vacuolate. They may be derived from cells in close association with the leaf trace or stipular bundles, or from cells in the region of a ray of the central cylinder.

The shapes and sizes of the proliferations are various, but tend toward a rounded, somewhat elongated structure, with a suggestion of a ring of differentiated cells, which in many cases are vascular—at least near the bundle with which union is established. They extend upward above the callus surface (fig. 23), laterally through the cortex, or laterally and downward in the cortex (fig. 22). The interpretation suggested is that they may have originated as unorganized meristematic masses of tissue, as in the 7-day-old tumor. Differentiation took place in the direction of a root primordium but did not progress far. With later growth of the tumor the cells did not remain meristematic, but most of them enlarged, became vacuolate, and a few became lignified, finally resulting in the rather unorganized masses of tissue seen in the two old tumors.

CYTOLOGICAL DETAILS

Almost without exception, nuclei of the endodermis, pericycle, cambium, and parenchymatous cells of the vascular bundle and of the cortex and pith in close association with the vascular tissue, and, with occasional exceptions, other cells of the cortex and pith, are of normal size. If in division, they show the diploid number of chromosomes, 12. The normal resting nucleus has two medium-sized nucleoli which may fuse to form one large one. The stages of mitosis all proceed in a normal manner. In a few instances, as already stated, nuclear division apparently is not accompanied by cell plate formation, thus giving rise to binucleate and, in a very few cases, multinucleate cells. In some of the large par-

enchymatous cells of the pith and cortex of both treated and control stems (although somewhat more frequently in the treated) large nuclei are found which may have one very large nucleolus or two large or four medium-sized ones. These number of polyploid cells, the fact that they are found in controls as well as in treated stems, and the regions in which they occur, do not as yet indicate any particular significance to the polyploid condition observed in this experiment.



FIGS. 22, 23.—Proliferations in old tumors. Fig. 22, 35 days old, diagonal section; portions of three proliferations at different distances from stipular bundle. Fig. 23, 46 days old, tangential section; region of cortex, stipular bundle on left. Mass at right establishes union with leaf trace. Portion of cross-section of root primordium at base.

facts, according to BERGER (4), suggest a tetraploid condition. Division figures found in comparable cells show more than the diploid number of chromosomes. In a very few instances the degree of polyploidy may be greater, but this is highly infrequent. These cells with tetraploid nuclei are few, are found in regions not extremely active, and appear to occur normally in the stems of control plants to a certain extent. So few were found that it cannot be said by what method doubling occurred. The small

Discussion

The responses just discussed enlarge upon and corroborate to a certain extent the work of LAIBACH (19) and SOLACOLU *et al.* (27) on *Vicia faba*. LAIBACH reports roots only in the largest callus proliferations formed on decapitated stems treated with indoleacetic acid in lanolin, while according to SOLACOLU *et al.* similarly induced tumors 9–10 days old show root meristems, completely individualized, producing rootlets with limited

growth and abnormal development. The latter find that the tumor extends much farther down the stem than here reported, and that meristematic activity, except in direct contact with the paste, is localized in the meristematic zones, the cambium and pericycle. The results of the present experiment do not agree completely with this. Activity in the cortex extends at least half the length of the older tumor, activity of the pith extends sometimes the complete length of the tumor, and the endodermis is more meristematic in all regions than is the pericycle. They report extreme activity near the surface in older tumors but give no account of the vascularization of the endodermal derivatives reported in the present paper. They do, however, describe many tracheids differentiated in various regions near the cut surface.

The responses of *V. faba* to indoleacetic acid show marked similarities to the responses of other plants to similar treatments, although there are certain differences, notably in degree of response. As stated by HAMNER and KRAUS (13) for kidney bean, the results of this experiment indicate that any parenchymatous tissue of the stem of broad bean may become meristematic in response to treatment with indoleacetic acid and these cells later differentiate into vascular tissues. The epidermis forms an exception to this, but in old, very active tumors of *Vicia*, some epidermal cells may undergo division.

The slight surface callus formation of *V. faba* when treated with indoleacetic acid is different from the reaction of kidney bean (13), cabbage (9), tobacco (11), and sunflower (5) to the same treatment, while resembling somewhat the response of tomato (6), *Iresine* (14), pea (26), and *Mirabilis* (12). The regions of greatest activity vary from plant to plant and also with the growth substance used. The extreme activity of the cortex of broad bean resembles that of the kidney bean treated with naphthaleneacetic acid, except for the vascularization (13). Meristematic activity of the endodermis is found in many of the plants that have been treated; it is more extended in kidney bean (2, 13, 17, 22) than in broad bean. Increased activity of the fascicular cambium and development of an interfascicular cambium as seen in broad bean are characteristic of tumors of *Mirabilis* (12), and to a certain extent other plants treated with indoleacetic acid; but greatest development is reached, particularly as regards production of secondary xylem, in tumors of kidney bean induced by naphthalene acetamide (18). The pericycle, which is rather inactive in broad bean, tomato (6), cabbage (9), kidney bean (13, 17), and sunflower (5), is a region of great activity in *Mirabilis* (12) and *Iresine* (14), and is relatively active in pea (26). The phloem, which in broad bean is relatively inactive, is very active in kidney bean (2, 13, 17), pea (26), *Iresine* (14), and tomato (6). Divisions in the pith, so marked in *Vicia* tumors, are also found in others, as in *Mirabilis* (12), to a limited extent in tomato (6), in the formation of the callus in kidney bean (13), in limited regions of cabbage (9), and in sunflower (5). In other plants the pith remains relatively inactive, except close to the primary xylem strands. The relative inactivity of the epidermis is characteristic of all plants treated with indoleacetic acid, except of laterally treated stems of *Iresine* (14).

Irregular differentiation of vascular tissues is found in many of the tumors, but vascularization to the extent and

regularity characteristic of *V. faba* is not found except in the kidney bean tumors induced by tryptophane (16). The similarity between these two tumors in this and other respects is particularly interesting and needs further detailed consideration.

The greatest difference between the tumors produced by *V. faba* after treatment with indoleacetic acid and similarly produced tumors on other plants is the almost complete lack of root initiation. Kidney bean tumors induced by tetrahydrofurfuryl butyrate (25) and tryptophane (16) also show no root formation, but in other indoleacetic acid-induced tumors root formation is extensive and a characteristic feature of the tumor. The rather unorganized proliferations of *Vicia* correspond to nothing reported for other plants, unless such proliferations are interpreted as roots. If this is done the problem remains as to why the cells should have become vacuolate and non-meristematic without complete differentiation and organization of tissues characteristic of roots. In other plants the root primordia, even though they may not develop far enough to break through the epidermis, remain meristematic and will resume activity if placed in a moist atmosphere. The occurrence of these structures leads to speculation as to the possibility of the existence of an organizing substance not present—or not produced in sufficient quantity—in *V. faba* for the development of normal roots.

The results of the present work, when compared with other similar work, would seem to suggest that both the degree and the type of response to any one growth-regulating substance, such as indoleacetic acid, varies with the species, each species offering a somewhat different internal environment. Certain of the investigations indicate that the effects vary in degree, particularly with variation in external environment, and in degree also with the age of the plant at time of treatment. This latter case has also proved true in *Vicia*.

Although polyploidy occurs to some extent in the tumors produced on the stem of *V. faba*, it seems improbable that it is specifically induced by the indoleacetic acid, although GREENLEAF (11) considers the polyploid condition of shoots produced by decapitated tobacco plants to be induced by this acid and LEVAN (20) finds polyploid cells in abundance in roots of onion treated with indoleacetic acid and other growth substances. DERMEN (8) reports polyploid cells in bean stems treated with naphthaleneacetic acid, but these are mainly in tissues likely to disintegrate subsequently. The potentiality for polyploidy is present normally in stems of *V. faba*, as shown by the scattered large nuclei and occasional polyploid figures in sections of control plants. It is conceivable that this tendency receives greater expression in treated plants because of the delay in maturation of and stimulation to increased activity in the cells in which the polyploidy occurs. A similar type of explanation might be extended to the case of tobacco, where callus does not normally form (10) but is induced by indoleacetic acid[1] and to the case of bean treated with naphthaleneacetic acid. The appearance of great numbers of polyploid cells in onion roots is more difficult to explain on a similar basis, although great numbers of such cells are found normally in roots of certain plants,

[1] Tomato produces polyploid shoots from a callus without the presence of the growth substance (21, 23).

for example, *Spinacia* (3), and moderate numbers in roots of certain legumes (28). However that may be, much more careful study is necessary before the specific relationship between growth-regulating substances and the possible induction of polyploidy can be established.

Summary

1. Plants of *Vicia faba* were decapitated just below the fifth node and the cut end treated with 1.5 per cent indoleacetic acid in lanolin or with lanolin alone.

2. The plants treated with indoleacetic acid responded by the formation of a tumor. The area, at most not more than 5 mm. long, below the treated surface became swollen and yellowed. There was little increase in size after the first 2 weeks following treatment.

3. The anatomy of the fourth internode of *V. faba* is similar to that in *Pisum sativum*, except for the number of bundles in the central cylinder.

4. Histologically every parenchymatous tissue became active to some extent in the formation of the tumor. Epidermal and outer cortical cells enlarged radially; the inner cortical cells divided transversely, occasionally the outer cortical cells tangentially; the endodermis divided tangentially; the cambium increased its activity and an interfascicular cambium was formed and became active as the early signs of response. Later parenchyma of the xylem, phloem, pericycle, pith, and rays became active to varying degrees. Cells of the pith divided repeatedly, and those bordering on the central cavity gave rise to an internal callus, the cells of which also divided repeatedly. In later stages all the cells of the cortex became highly meristematic. In some cases a callus was formed above the cut surface, starting from active cells of the vascular region and added to eventually by all parenchymatous cells. In the oldest tumors an occasional tangential division of the epidermis may be found.

5. Vascularization of derived tissues is somewhat extensive. Particularly notable is the response of the endodermal region of the central cylinder and of the cortical bundles. This group of extravascular strands may be supplemented by an occasional one formed in the pericycle or phloem. Small groups of wound tracheids are differentiated in the pith, pith callus, cortex, and surface callus when present. Occasionally vascular strands are formed in the pith close to the protoxylem points of the regular vascular cylinder.

6. Root induction, characteristic of some other plants treated with indoleacetic acid, is uncommon in *V. faba* under the conditions of this experiment. Those formed are probably derived as described for other plants. Occasionally, however, large, rather unorganized masses of tissue are developed which are probably best interpreted as potential roots incompletely organized.

7. Occasional polyploid nuclei are found in the large parenchymatous cells of the cortex and pith in both treated and control stems of *V. faba*, although somewhat more numerously in the treated stems. All divisions appear to be regular.

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LITERATURE CITED

1. BEAL, J. M., Histological responses of three species of *Lilium* to indoleacetic acid. BOT. GAZ. **99**:881–911. 1938.
2. ———, Effect of indoleacetic acid on thin sections and detached segments of the second internode of the bean. BOT. GAZ. **102**:366–372. 1940.
3. BERGER, C. A., Reinvestigation of polysomaty in *Spinacia*. BOT. GAZ. **102**:759–769. 1941.
4. ———, Some criteria for judging the degree of polyploidy of cells in the resting stage. Amer. Nat. **75**:93–96. 1941.
5. BLUM, J. L., Responses of sunflower stems to growth-promoting substances. BOT. GAZ. **102**: 737–748. 1941.
6. BORTHWICK, H. A., HAMNER, K. C., and PARKER, M. W., Histological and microchemical studies of the reactions of tomato plants to indoleacetic acid. BOT. GAZ. **98**:491–519. 1937.
7. COMPTON, R. H., An investigation of the seedling structure in the Leguminosae. Jour. Linn. Soc. Bot. **41**:1–122. 1912.
8. DERMEN, HAIG, Intranuclear polyploidy in bean induced by naphthalene-acetic acid. Jour. Hered. **32**:133–138. 1941.
9. GOLDBERG, ETHEL, Histological responses of cabbage plants grown at different levels of nitrogen nutrition to indole(3)acetic acid. BOT. GAZ. **100**:347–369. 1938.
10. GREENLEAF, W. H., Induction of polyploidy in *Nicotiana*. Science **86**:565–566. 1937.
11. ———, Induction of polyploidy in *Nicotiana* by hetero-auxin treatment. Jour. Hered. **29**:451–464. 1938.
12. HAMNER, K. C., Histological responses of *Mirabilis jalapa* to indoleacetic acid. BOT. GAZ. **99**: 912–954. 1938.
13. HAMNER, K. C., and KRAUS, E. J., Histological reactions of bean plants to growth-promoting substances. BOT. GAZ. **98**:735–807. 1937.
14. HARRISON, B. F., Histological responses of *Iresine lindenii* to indoleacetic acid. BOT. GAZ. **99**:301–338. 1937.
15. HAYWARD, H. E., The structure of economic plants. New York. 1938.
16. KRAUS, E. J., Histological reactions of bean plants to l-tryptophane. BOT. GAZ. **102**:602–622. 1941.
17. KRAUS, E. J., BROWN, NELLIE A., and HAMNER, K. C., Histological reactions of bean plants to indoleacetic acid. BOT. GAZ. **98**:370–420. 1936.
18. KRAUS, E. J., and MITCHELL, J. W., Histological and physiological responses of bean plants to alpha naphthalene acetamide. BOT. GAZ. **101**: 204–225. 1939.
19. LAIBACH, F., Über die Auslösung von Kallus- und Wurzel-bildung durch β-Indolylessigsäure. Ber. deutsch. Bot. Ges. **53**:359–364. 1935.
20. LEVAN, ALBERT, Cytological phenomena connected with the root swelling caused by growth substances. Hereditas **25**:87–96. 1939.
21. LINDSTROM, E. W., and KOOS, KATHARINE, Cyto-genetic investigations of a haploid tomato and its diploid and tetraploid progeny. Amer. Jour. Bot. **18**:398–410. 1931.
22. LINK, G. K. K., WILCOX, HAZEL W., and LINK, ADELINE DES., Responses of bean and tomato to *Phytomonas tumefaciens*, *P. tumefaciens* extracts, β-indoleacetic acid, and wounding. BOT. GAZ. **98**:816–867. 1937.
23. MATHER, K., The origin of tetraploid shoots from the callus of diploid tomatoes. Jour. Genet. **27**:127–132. 1933.
24. MULLER, CAMILLE, La tige feuillée et les cotylédons des Viciées à germination hypogée. La Cellule **46**:195–354. 1937.
25. MULLISON, W. R., Histological responses of bean plants to tetrahydrofurfuryl butyrate. BOT. GAZ. **102**:373–381. 1940.
26. SCOTT, FLORA M., Anatomy of auxin treated etiolated seedlings of *Pisum sativum*. BOT. GAZ. **100**:167–185. 1938.
27. SOLACOLU, THÉODORE, CONSTANTINESCO, DÉMETRE, and CONSTANTINESCO, MARGUERITE, Etude anatomique et cytologique des modifications provoquées par les substances organoformatives, sur les tiges décapitées de *Vicia faba* L. Compt. rend. Acad. Sci. Paris **206**: 1985–1987. 1938.
28. WIPF, LOUISE, and COOPER, D. C., Somatic doubling of chromosomes and nodular infection in certain Leguminosae. Amer. Jour. Bot. **27**: 821–824. 1940.

STUDIES IN CALIFORNIAN HEPATICAE
III. CRYPTOMITRIUM TENERUM

ARTHUR W. HAUPT

(WITH TWENTY-EIGHT FIGURES)

Introduction

Cryptomitrium tenerum (Hook.) Aust. is said by EVANS (4) to occur in "California and Mexico; probably also in Chili." Since this was written, the species has been reported from Washington by CLARK and FRYE (2), while more recently it has been collected by the writer (9) in Costa Rica and Guatemala. Its occurrence in South America is doubtful, being based on its supposed identity with *Duvalia brevipedunculata* Mont. from Chili, described nearly a century ago. *Cryptomitrium* was long thought to be monotypic, but a second species, *C. himalayense*, has been described from northern India by KASHYAP (11).

Cryptomitrium tenerum is widely distributed in California, but, at least in the southern part of the state, seems nowhere to be locally abundant. It has been collected by the writer in the Santa Monica, San Gabriel, and Cuyamaca Mountains at relatively low elevations, mostly below 2000 feet, where it grows in ravines on moist shaded banks. It usually reaches its best development in February or March, depending on the amount and distribution of the winter rainfall. During the long rainless season the plants dry up, the tips reviving and giving rise to new plants with the advent of the autumn rains.

Material for the present study was collected both in southern California and in Costa Rica. No differences were observed between the plants obtained from these two widely separated regions. They undoubtedly belong to the same species. The superficial features of *C. tenerum* are well described and illustrated by HOWE (10). The only morphological investigation that has been made is by ABRAMS (1). *Cryptomitrium* belongs to that section of the Marchantiaceae designated by LEITGEB (12) as the Operculatae and which includes the well-known genera *Plagiochasma*, *Grimaldia*, *Reboulia*, and *Asterella*. *Cryptomitrium* is most closely related to *Grimaldia*, differing from it chiefly in having an almost flat rather than a strongly convex carpocephalum and a bilabiate rather than an undivided involucre.

Observations

THALLUS

The thallus is pale green and rather thin and delicate. Its branching is mainly dichotomous but sometimes occurs by means of either apical innovations or ventral adventitious shoots. The epidermal cells, forming a single layer, have slightly thickened walls and many chloroplasts. The air pores are small, simple, and with unthickened radial walls. The air chambers, occurring in several layers, are separated by partitions one cell thick. They are sparingly subdivided by supplementary partitions. Special chlorophyllose filaments are wanting. Chloroplasts are abundant in the cells of the loose dorsal region and also occur in those lying immediately below. The ventral

region is composed of compactly arranged, thin-walled cells. No mucilage cells and no endophytic fungus were seen in the material studied. Oil cells are of infrequent occurrence. The ventral scales, arranged in two longitudinal rows, have filiform appendages. Both smooth-walled and tuberculate rhizoids are present.

Growth of the thallus results from the activity of a single apical cell of the

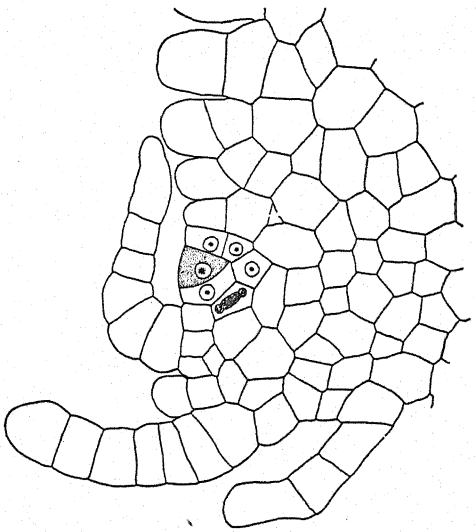


FIG. 1.—Median longitudinal section through apical cell showing schizogenous origin of air chambers behind it. ×450.

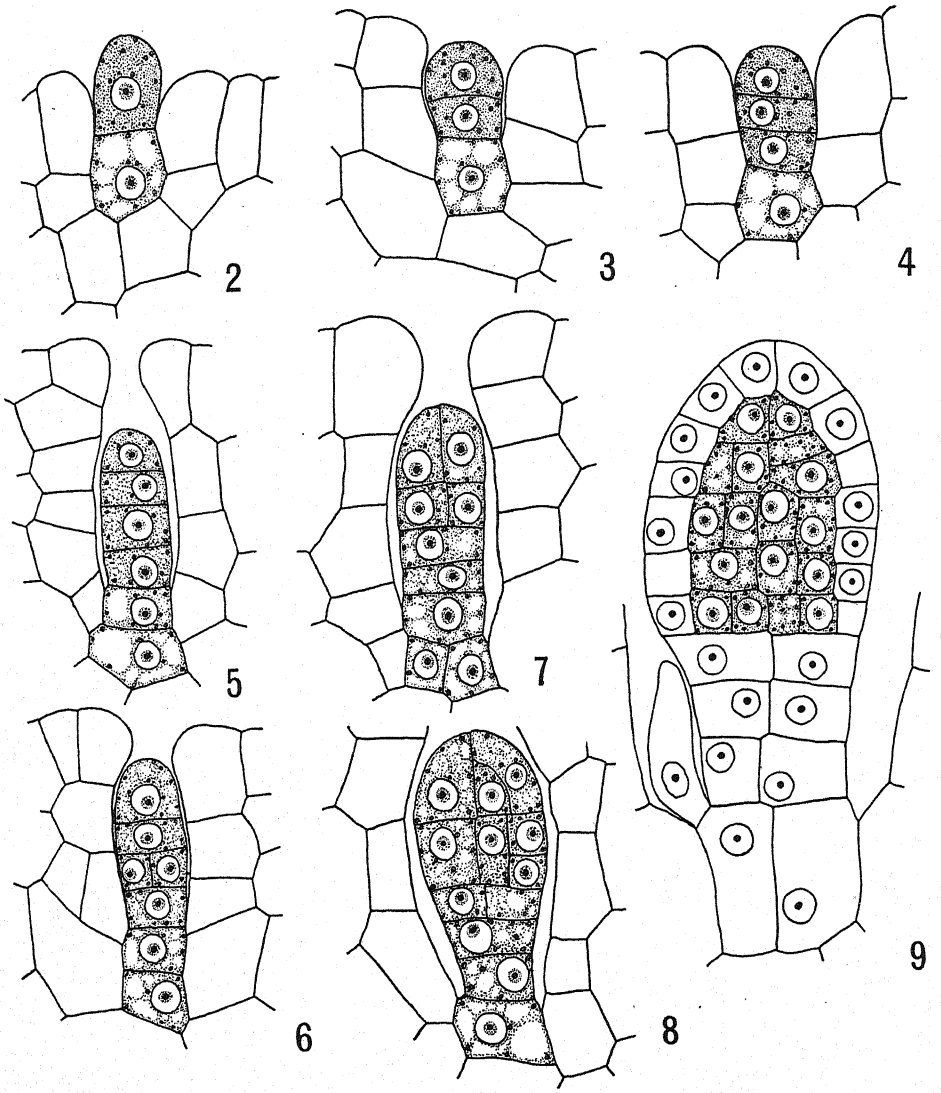
cuneate type, characteristic of all the Marchantiales (fig. 1). The air chambers originate close behind the apical cell by intercellular cleavage, the split beginning at the surface and progressing inward, as in *Reboulia* (5) and *Asterella* (8). The air chambers are deepened by upgrowth of the surrounding tissue.

Cryptomitrium tenerum is monoecious. As in certain species of *Grimaldia* and *Asterella*, the antheridia form a narrow, elongated, median, dorsal group lying immediately posterior to the female receptacle. The pore communicating with the antheridial chamber is considerably raised above the general level of the thallus. The air pores communicating with the surrounding air chambers are simple, as in the other genera of Operculatae.

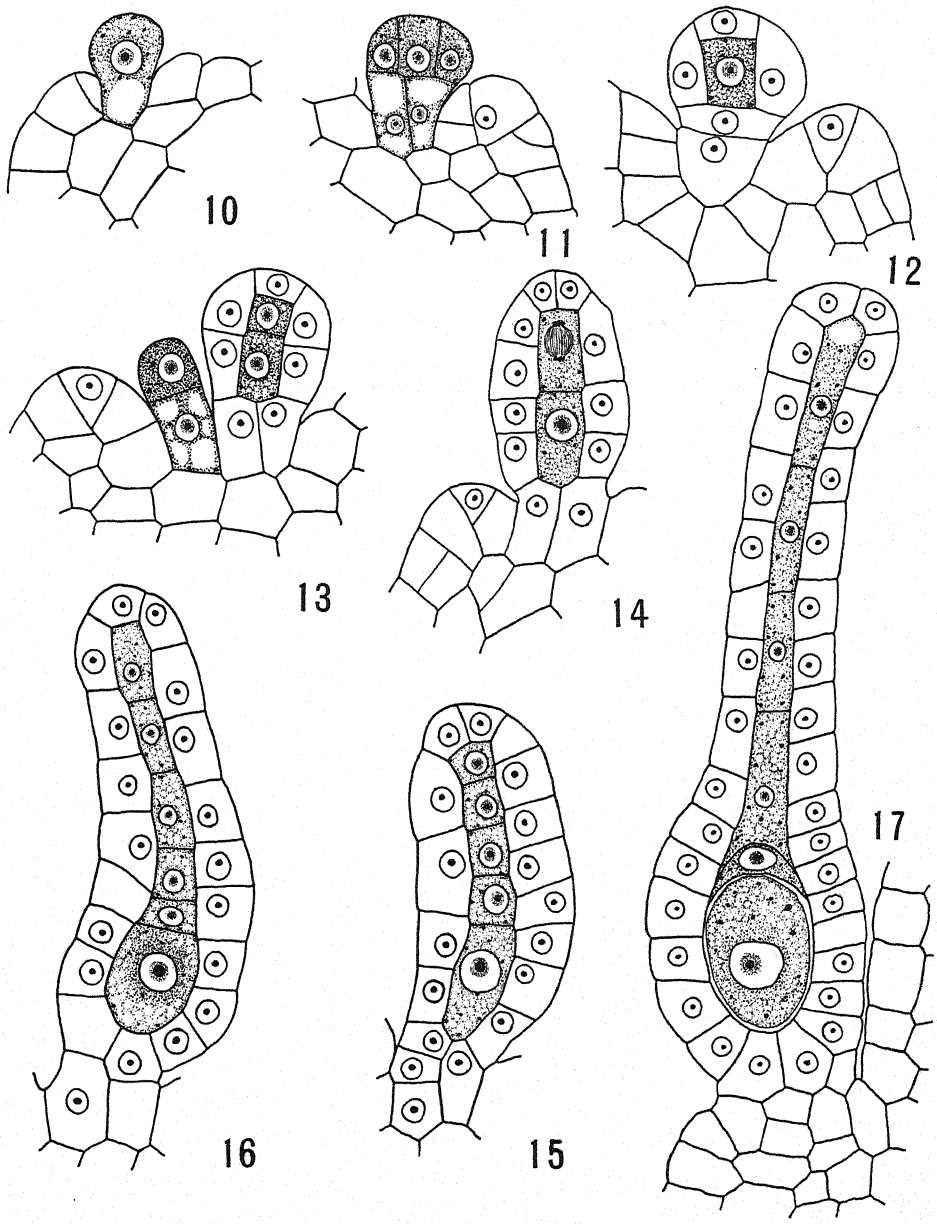
The female receptacle is terminal. Its stalk has a single rhizoid furrow. The disk is hemispherical when young but finally becomes circular and nearly flat. It is without lobes when mature. ABRAMS (1) has demonstrated that the young receptacle has five growing points (rarely four) and thus represents a branch system. The apical cell of the thallus is carried upward on the receptacle and soon undergoes two successive dichotomies. Of the four growing points thus established, one usually divides again to form five in all. Between the growing points lies a rhizoid furrow continuous with the furrow of the receptacle stalk. Three or four archegonia arise in acropetal succession immediately behind each growing point. They become surrounded by a bilabiate involucre. The activity of the apical cells on the carpocephalum is checked by the formation of the archegonia, further growth being intercalary. As in the other genera of Operculatae, the air pores on the female receptacle are compound (barrel-shaped) but elsewhere are simple. The air chambers on the female receptacle are unusually extensive. They form a single layer.

ANTHERIDIUM.—The antheridia arise in acropetal succession from immediate dorsal segments of the apical cell. They become sunken in individual pits in the thallus formed by upgrowth of the surrounding tissue. The papillate initial undergoes a transverse division into an inner and an outer cell (fig. 2). Generally three additional transverse divisions in the outer cell result in the formation of a filament of four cells in the superficial portion of the antheridium before any

vertical divisions take place (figs. 3–5). Vertical walls appearing at right angles to each other are developed in the three uppermost cells, while another transverse wall may be formed in one of the cells below (figs. 6, 7). Periclinal walls arising in the three uppermost tiers now cut off the outer sterile jacket from the central spermatogenous cells (figs. 8, 9). The lower cells form the stalk, which in the mature antheridium becomes rather thick. As in *Reboulia*, unicellular hairs arise at the



FIGS. 2–9.—Stages in development of antheridium: fig. 2, first division of antheridium initial; figs. 3–5, division of outer cell to form filament of four cells; figs. 6, 7, formation of vertical walls; figs. 8, 9, formation of periclinal walls. ×900.



FIGS. 10–17.—Stages in development of archegonium: fig. 10, archegonium initial; fig. 11, formation of primary axial cell and primary wall cells; fig. 12, formation of central cell and cover cell; fig. 13, first division of archegonium initial (left) and formation of primary ventral cell and primary neck canal cell (right); fig. 14, division of primary neck canal cell; fig. 15, formation of four neck canal cells; fig. 16, differentiation of ventral canal cell and egg from primary ventral cell; fig. 17, nearly mature archegonium. ×600.

base of the young antheridium and secrete mucilage into the antheridial chamber (fig. 9).

The development of the antheridium follows the usual pattern that has been described in *Reboulia* (5), *Asterella* (8), *Preissia* (7), and other Marchantiales. ABRAMS (1) found in *Cryptomitrium* that the outer cell resulting from the transverse division of the antheridium initial gives rise to a row of three rather than of four cells before any vertical walls are developed. This condition was observed in two instances during the present study but is certainly not the usual one. ABRAMS found also that the uppermost cell of the young antheridium does not contribute to the formation of the spermatogenous tissue, a feature with which the present investigation is in disagreement, as figure 8 clearly indicates.

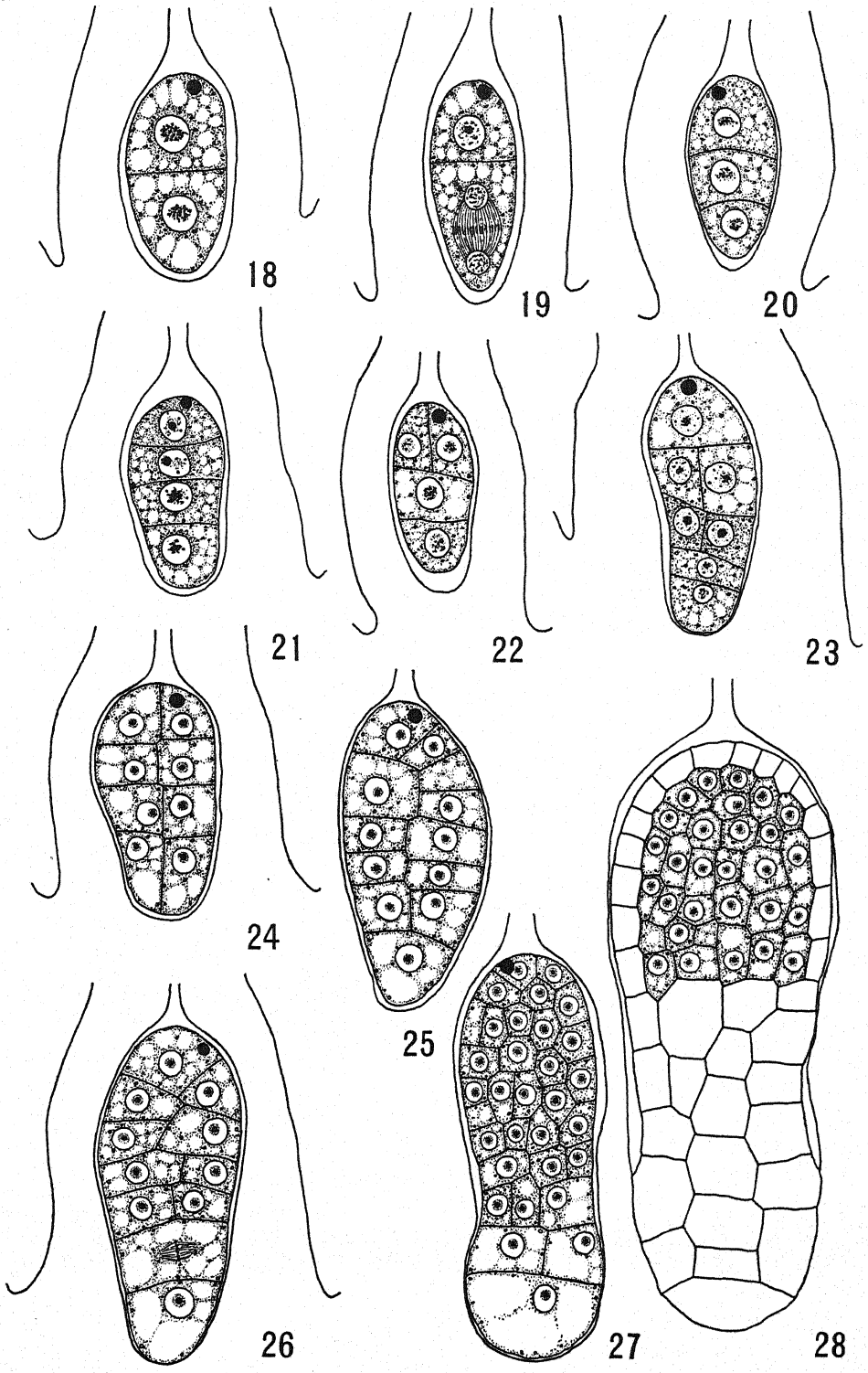
ARCHEGONIUM.—The archegonia appear on the carpocephalum only after it has become conspicuously dome-shaped, arising very close to the apical cell. ABRAMS traced the development of the archegonium and found that the outer cell arising from a transverse division of the papillate initial undergoes the usual three vertical divisions that differentiate the primary axial cell from the primary wall cells. These stages have been confirmed by the present investigation (figs. 10, 11). The primary axial cell gives rise to the cover cell and central cell (fig. 12), the central cell to the primary neck canal cell and primary ventral cell (fig. 13), and the primary ventral cell to the ventral canal cell and egg (fig. 16). The ventral canal cell is small. A vertical wall is formed in the cover cell before the primary neck canal cell divides (fig. 14). ABRAMS observed that this vertical wall does not appear until some time after four neck canal cells have been formed. He also found that "the usual number of neck canal cells is eight."

In all the preparations used in the present study, four neck canal cells are derived from the primary neck canal cell before the primary ventral cell divides to form the ventral canal cell and egg (figs. 14–16). Ordinarily, with the increase in length of the neck and rounding up of the egg, the number of neck canal cells remains four (fig. 17). In many archegonia approaching maturity, however, some or all of the neck canal cells become binucleate. In *Reboulia* (5) the ventral canal cell and egg are differentiated after four neck canal cells have been formed, but this number is later increased to eighteen or twenty. In *Asterella* (8) the situation is similar, except that only eight neck canal cells are eventually produced.

The first archegonia reach maturity while the carpocephalum is still small, its development continuing as additional archegonia are formed. The neck of the mature archegonium hangs straight downward and does not, like that of *Reboulia*, curve outward and upward. It is often slightly twisted. The stalk of the carpocephalum begins to elongate as soon as fertilization has taken place, carrying the embryos upward. Although more than one young sporophyte may be seen in each archegonial group, generally only one (rarely two) reaches maturity. Some of the jacket cells of the venter divide periclinally before fertilization, while after fertilization the venter forms a calyptra that becomes three to five layers of cells thick.

EMBRYO

The first division of the fertilized egg is transverse, forming two segments approximately equal in size (fig. 18). ABRAMS (1) claims that the embryo of



FIGS. 18–28.—Stages in development of embryo: fig. 18, fertilized egg divided transversely; figs. 19, 20, transverse division of hypobasal segment; fig. 21, same of epibasal segment; fig. 22, vertical division of epibasal segment (unusual condition); fig. 23, same of two middle cells and transverse division of basal cell; fig. 24, vertical division of all four cells; figs. 25, 26, older stages with triangular cell at apex and undivided cell at base; fig. 27, older stage showing growth of foot, seta, and capsule; fig. 28, still older stage showing differentiation of sporogenous tissue. ×470.

Cryptomitrium displays an octant stage, but the writer has seen only filamentous embryos. The second division is always parallel with the first one and occurs in the hypobasal segment (figs. 19, 20). Subsequent behavior strongly indicates that, of the three cells formed, the upper one eventually gives rise to the capsule, the middle one to the seta, and the lower one to the foot. Sometimes a vertical division may now occur in the upper cell (fig. 22), but more commonly a transverse division takes place instead, thus forming a filament of four cells (fig. 21). Vertical walls at right angles to each other are then developed in each tier, the four tiers thus consisting of four cells each (fig. 24). This stage was seen frequently. Occasionally an additional transverse division occurs in the basal cell before the formation of vertical walls is complete (fig. 23).

Following the appearance of vertical walls in all four tiers, additional transverse and vertical walls come in somewhat irregularly, especially in the upper two-thirds of the embryo (fig. 27). Few divisions take place in the foot at this stage. Often the walls in the uppermost tier are more or less oblique, so that the embryo seems to have an apical cell, and this may appear to cut off one or more segments (figs. 25, 26). The formation of periclinal walls in the capsular region results in the delimitation of an outer layer of sterile cells from a central mass of sporogenous tissue (fig. 28). Until this stage is reached, a large oil globule is nearly always found at the upper end of the embryo, as in *Reboulia*. In the mature egg several smaller oil globules are generally present.

The early embryogeny of *Cryptomitrium* is similar to that of *Reboulia* as described by WOODBURN (18), HAUPT (6), and DUPLER (3), whose accounts differ chiefly in the interpretation of certain stages found by all. A filamentous embryo consisting of four superimposed cells is formed before the appearance of vertical walls. Both WOODBURN and HAUPT thought that the second wall is developed in the epibasal segment, but DUPLER showed by mitotic figures that the second division occurs in the hypobasal segment and the third in the epibasal segment, a sequence which the present study has demonstrated for *Cryptomitrium*. Furthermore, it seems probable that in *Reboulia*, as DUPLER has suggested, the epibasal cell gives rise to the capsule and the hypobasal cell to the foot and seta. Both WOODBURN and DUPLER observed that the upper cell and occasionally the basal cell of the vertical row of four may divide obliquely to form a triangular cell. DUPLER claimed that the upper one may function as an apical cell.

The writer (8) has shown that in *Asterella*, as in *Reboulia*, a filament of four cells is formed before any vertical walls appear. Here also it seems likely that a transverse division occurs in both the hypobasal and epibasal segment, the lowest cell probably giving rise to the foot, the next one to the seta, and the two upper ones to the capsule. In both *Plagiochasma* and *Grimaldia*, MEYER (13, 15) has shown that filamentous embryos are produced. In *Grimaldia* the earliest stages were not seen, but in *Plagiochasma* both the hypobasal and epibasal segments undergo transverse division, forming a filament of four cells in which vertical walls are subsequently developed in the usual way. In both genera MEYER observed slightly older embryos with a triangular "apical cell" that seems to cut off a few segments.

In addition to the five genera of Operculatae, filamentous embryos have been observed in *Targionia* by O'KEEFFE (17) and in *Conocephalum* (*Fegetella*) by MEYER (14). In a discussion of the phylogeny

of the Marchantiales, MEYER (16) regards the filamentous type of embryo as more primitive than the octant type, such as occurs in *Riccia*, *Oxymitra*, *Corsinia*, *Clevea*, *Preissia*, and *Marchantia*.

OLDER SPOROPHYTE

The further growth of the capsule and foot greatly exceeds that of the seta, and as a result the young sporophyte assumes a dumb-bell shape. This is related to the fact that the embryo, filamentous at first, soon becomes considerably elongated. The same condition is seen in the other genera of Operculatae, as well as in *Targionia* and *Conocephalum*. A dumb-bell form is not assumed by the young sporophyte of *Preissia* and *Marchantia*, forms in which the embryo exhibits an octant stage and becomes globular (or but slightly elongated) as further growth and differentiation take place.

The development of the sporogenous tissue in *Cryptomitrium* and its differentiation into spore mother cells and elaters follow the same pattern as that described for *Reboulia* (6). In both genera the spore mother cells and elaters are derived from the sporogenous cells by the same number of cell divisions, and so each elater is homologous with a single spore mother cell. The mature sporophyte has a bulbous foot, a very short seta, and a globular capsule. The capsule wall is one-layered and without annular thickenings, except at the apex, where it consists of two layers, the inner one having much larger cells than the outer one and bearing a few thickenings. The apex of the capsule forms a distinct operculum that remains intact in dehiscence. The elaters have two spiral bands of thickening.

Summary

1. The thallus of *Cryptomitrium tenerum* grows by means of a cuneate apical cell, behind which the air chambers arise by intercellular splitting that begins at the surface.

2. The antheridia are borne in an irregular median dorsal group, the archegonia in a terminal receptacle representing a branch system with usually five growing points, behind each of which three or four archegonia arise in acropetal succession.

3. The air pores on the female receptacle are compound but elsewhere are simple.

4. The antheridia develop in a manner that is typical for the Marchantiales. The formation of periclinal walls delimiting the spermatogenous tissue involves the three uppermost tiers of cells of the young antheridium.

5. In the development of the archegonium four neck canal cells are formed before the ventral canal cell and egg are differentiated. Some or all of the neck canal cells may later become binucleate.

6. A filamentous embryo consisting of four superimposed cells is formed before the appearance of vertical walls.

7. After the first transverse wall has appeared in the fertilized egg, both segments undergo transverse division, that of the hypobasal segment occurring first.

8. Of the two cells derived from the hypobasal segment, the lower one eventually gives rise to the foot and the upper one to the seta, while the two cells derived from the epibasal segment form the capsule.

9. The early embryogeny of *Cryptomitrium* closely resembles that of *Reboulia*, *Asterella*, and *Plagiochasma*.

10. An elater in *Cryptomitrium* is homologous with a single spore mother cell, not with a row of them.

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LITERATURE CITED

1. ABRAMS, LEROY, The structure and development of *Cryptomitrium tenerum*. BOT. GAZ. **28**: 110–121. 1899.
2. CLARK, LOIS, and FRYE, T. C., The liverworts of the Northwest. Publ. Puget Sound Biol. Sta. **6**:1–193. 1928.
3. DUPLER, A. W., Early embryogeny of *Reboulia hemisphaerica*. BOT. GAZ. **74**:143–157. 1922.
4. EVANS, A. W., Marchantiales. In North American Flora **14**[1]:9–66. 1923.
5. HAUPT, A. W., Gametophyte and sex organs of *Reboulia hemisphaerica*. BOT. GAZ. **71**:61–74. 1921.
6. ———, Embryogeny and sporogenesis in *Reboulia hemisphaerica*. BOT. GAZ. **71**:446–453. 1921.
7. ———, Morphology of *Preissia quadrata*. BOT. GAZ. **82**:30–54. 1926.
8. ———, Studies in Californian Hepaticae. I. *Asterella californica*. BOT. GAZ. **87**:302–318. 1929.
9. ———, Notes on some Hepaticae of Costa Rica. Bryologist **45**:138–146. 1942.
10. HOWE, M. A., The Hepaticae and Anthocerotes of California. Mem. Torr. Bot. Club **7**:1–208. 1899.
11. KASHYAP, S. R., Morphological and biological notes on new and little known West-Himalayan liverworts. III. New Phytol. **14**:1–18. 1915.
12. LEITGEB, H., Untersuchungen über die Lebermoose. VI. Die Marchantieen. Graz. 1881.
13. MEYER, K. I., Untersuchungen über den Sporophyt der Lebermoose. II. Die Entwicklungsgeschichte des Sporogons bei *Plagiochasma*. Bull. Soc. Imp. Nat. Moscow **27**:597–615. 1913.
14. ———, Die Entwicklung des Sporogons bei *Fegetella conica*. Planta **8**:36–54. 1929.
15. ———, Zur Entwicklungsgeschichte des Sporophyten einiger Marchantiales. Planta **13**:193–209. 1931.
16. ———, Die Sporophytenentwicklung und die Phylogenie bei den Marchantiales. Planta **13**: 210–220. 1931.
17. O'KEEFFE, LILLIAN, Structure and development of *Targionia hypophylla*. New Phytol. **14**:105–116. 1915.
18. WOODBURN, W. L., Preliminary notes on the embryology of *Reboulia hemisphaerica*. Bull. Torr. Bot. Club **46**:461–464. 1920.

SPINDLE ABNORMALITIES IN MENTHA[1]

C. P. SWANSON AND RAY NELSON

(WITH TWENTY FIGURES)

Introduction

Various environmental agents (9, 13, 23) and meiotic studies of hybrids and mutants (3, 6, 22) have shown that the phenomenon of cell division, whether mitotic or meiotic, is a highly complex and delicately balanced one, a composite of several separate but integrated parts. That the study of these parts, made possible through a differential breakdown of the system, is far from complete, however, is evident from SCHRADER'S recent review of mitosis (17). No theory yet proposed adequately covers all the phenomena observed. The present study deals with a genetically controlled breakdown in the development and behavior of the spindle in the genus *Mentha*.

The material employed was derived from a group of species and varieties of *Mentha* used in a disease-resistance breeding program. The plants consisted of clonal lines of the peppermint, *Mentha piperita* L. var. Mitcham, the spearmint, *M. spicata* L. (Huds.) var. *crispata*, together with six of their interspecific hybrids. Scarcity of meiotic material permitted a detailed study of only the parental species and two of the hybrids, although small samples of meiotic cells were obtained from all. No mitotic cells were studied. The spindle stained well with acetocarmine; thus it was possible to study accurately the form and structure of this portion of the division figure.

[1] Journal article no. 590, new series; Michigan Agricultural Experiment Station.

Observations

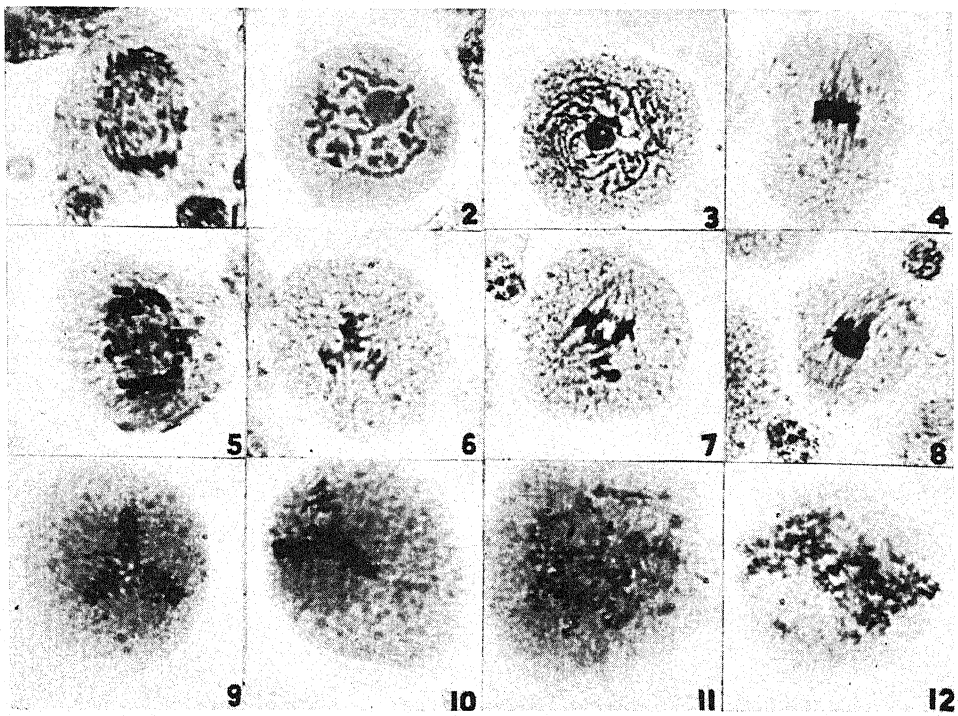
MEIOSIS IN GENERAL

Mentha piperita var. Mitcham is generally considered an interspecific hybrid, its suspected parents being *M. spicata* and *M. aquatica*. Its meiotic behavior is indicative of its hybrid origin. Almost completely pollen sterile, it sets little or no seed when selfed, even under the most favorable conditions. In crosses with *M. spicata* var. *crispata* some seed is produced, indicating that an occasional embryo develops. RUTTLE (12) has reported the diploid chromosome number as 65–69. The inability to distinguish between univalents and small bivalents, despite clear diakinetic figures, prevented accurate counts, although the number must be approximately 70. Considerable homologous pairing was evident, metaphase figures showing many bivalents. Pachytene stages showed unpaired threads and what appeared to be inversion loops. Diplotene and diakinesis stages were clear and well defined. Univalents were seen at metaphase, and at anaphase both lagging chromosomes and chromatin bridges were frequent (fig. 1). No spindle abnormalities were found, despite its considerable structural hybridity.

M. spicata var. *crispata* revealed no prophase irregularities of any kind. Pachytene stages showed numerous distinct chromatic knobs along the paired chromosomes (fig. 3). Diplotene, metaphase, and anaphase configurations (figs. 2, 4, 5), with the exception of spindle ab-

normalities, were regular, differing from SCHURHOFF's (19) observations which led him to conclude that this species is a hybrid. It is probable that the plant investigated by SCHURHOFF was a genetically distinct strain, since other known strains of this species are highly sterile. Diplotene stages revealed the beginnings of major coil formation (fig. 2), and the coiling cycle followed the *Tradescantia* type of behavior (20). Normal metaphases presented a compact, distinctly outlined spindle, but 50 per cent of the cells revealed a breakdown of the regular spindle mechanism. This breakdown was expressed in varying degrees and seemed to take no particular direction. It might be supposed that this would lead to considerable pollen abortion, but 85 per cent of the pollen appeared viable.

The *piperita* × *spicata* hybrids, six in number, were produced by encircling a bed of *M. piperita* with plants of *M. spicata* var. *crispata* and allowing bees to effect pollination. Seeds were taken only from *M. piperita*. That the seedlings are true hybrids is evident from their intermediate morphology. In oil properties, however, the peppermint characteristics strongly dominate. In the pollen mother cells, the hybrids show the chromosome irregularities of *M. piperita* (fig. 5) and the spindle abnormalities of *M. spicata*. All the hybrids revealed evidences of



FIGS. 1–12.—Fig. 1, *M. piperita* var. Mitcham; anaphase. Fig. 2, *M. spicata* var. *crispata;* diplotene. Fig. 3, same; pachytene. Fig. 4, same; metaphase. Fig. 5, *piperita* × *spicata* hybrid; anaphase. Figs. 6, 7, *M. spicata;* tripolar spindles. Fig. 8, same; curved open spindle. Fig. 9, *piperita* × *spicata* hybrid; tripolar spindle. Fig. 10, same; metaphase-anaphase. Fig. 11, same; abortive spindle. Fig. 12, same; small abortive spindle.

spindle breakdown, but the degree of expression varied from plant to plant.

Very little functional pollen was produced by *M. piperita* or its hybrids. RUTTLE (12) has stated that the whole genus is characterized by high pollen abortion. Pollen degeneration begins shortly after the quartet stage, and only rarely was an anther sufficiently full of viable pollen properly to dehisce.

GENETIC CONTROL OF SPINDLE DEVELOPMENT

Several instances of genetic control of spindle development and behavior have been reported. BEADLE (1) found in maize a recessive gene for producing supernumerary mitoses following meiosis; CLARK (3) has shown, again in maize, that the gene *dv*, when homozygous, produces a divergent spindle in the meiotic and gametophytic divisions; DARLINGTON and THOMAS (6) have described a *Festuca-Lolium* hybrid with aberrant spindles; and UPCOTT (21) has shown that pollen sterility in *Lathyrus* is due to a recessive gene which produces a timing upset in spindle development. In animals, WALD (22) has reported the abnormal effect of the mutant gene *claret* in *Drosophila simulans* on maturation and cleavage divisions.

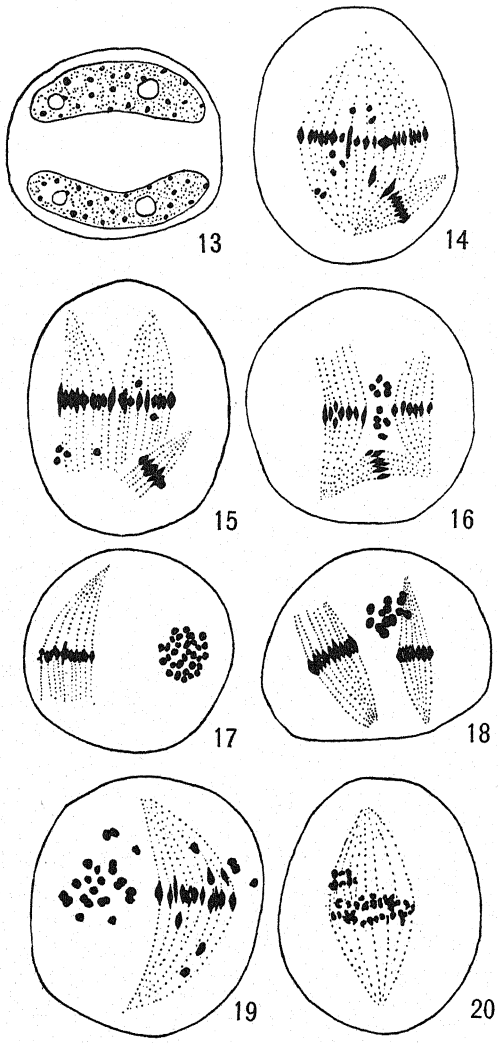
In the present study, the exact genetic control is still to be worked out. The data, however, indicate that the effect is a partially dominant one, since it has been transferred from a stable species, *M. spicata*, to all its hybrid offspring. It is known that the hybrid genotype is less well protected than the so-called "good" species against environmental changes; but that the spindle aberrancies are due to a definite genic control and not to hybridity or to a general gene unbalance is indicated by the derivation of the effect from the more regular of the two parents in meiosis, and by the fact that the hybrids show a lower percentage of abnormal cells than did *M. spicata*. The chromosomal unbalance contributed by *M. piperita* had no additive effect, in so far as the spindle is concerned. The genic control is not dominant in the sense that the effect is shown in every cell, however, but the degree of expression, or penetrance, of the presumably heterozygous condition in the hybrids is less than that of the original species, *M. spicata*. A varying percentage of the cells show spindle abnormalities, and these very irregularly. Within the same bud some anthers are completely normal, while others are equally abnormal; usually the anthers contain a mixture of normal and abnormal cells. Environmental conditions, both internal and external, appear to be the deciding factors, and since—as will be shown later—the spindle abnormalities take no specific form, what appears to be transmitted from *M. spicata* to its hybrid offspring is a susceptibility to environmental changes expressed through an instability of spindle form. There appears to be no directive control of spindle shape and behavior such as CLARK (3) has shown in the case of the divergent spindles in maize.

STRUCTURAL AND TIME VARIATIONS IN SPINDLE DEVELOPMENT

Variation in spindle development can be shown in two ways: (*a*) a structural variation expressing itself through deviations in the polar conditions as well as in spindle form and number; (*b*) a time variation expressing itself through lack of co-ordination with other nuclear processes, notably the chromosome spiralization cycle.

Mentha spicata, the male parent, showed the greatest percentage of cells with spindle upsets, but the range of vari-

ation was narrow. Only tripolar (figs. 6, 7) and curved open spindles (fig. 8) were found. The extra pole was either equal in size and structure to the other two (fig. 6), or it was small and abortive (fig. 7), merely distorting the spindle.



FIGS. 13–20.—Figs. 13–16, *piperita* × *spicata* hybrids; complete chromosome complement not shown (13, interphase; 14, accessory spindle at metaphase I; 15, 16, accessory spindles with central spindle divided). Figs. 17–20, *piperita* × *spicata* hybrids; complete chromosome complement not shown (17, metaphase II with curved open spindle; 18, metaphase I with two independent spindles; 19, metaphase I, curved spindle; 20, early anaphase).

It would be expected that tripolar spindles would lead to the formation of three nuclei at anaphase, as DARLINGTON and THOMAS (6) have shown; but such has not been found, although many cells were examined with this in mind. That no more than two nuclei are formed is probably due to the size and shape of the interphase nucleus (fig. 13), the long kidney-shaped structure occupying nearly the whole of one hemisphere of the cell, with its ends closely approximating those of its sister nucleus. Any extra nuclei which might be formed as a result of a tripolar condition would be incorporated into the larger nucleus, which, so far as the present observations show, forms as a rounded nucleus at telophase and then enlarges and spreads laterally, following the curvature of the cell walls.

The curved spindles were of the open type (fig. 8). They appeared to be too narrow and too long to be accommodated within the confines of the cell; hence they bend with the cell wall. The chromosomes seem to have no difficulty in reaching the indefinite polar regions to form a single regular nucleus. In this respect the spindles were similar to the divergent spindles in maize (3), except that only single telophase nuclei were formed. DOBZHANSKY (7) has described similarly curved spindles in *Drosophila*, but which possessed clearly differentiated polar regions.

The clonal line of *M. spicata* used in this study is a highly fertile one, in contrast to nearly all the varieties and species of this genus (12). Apparently, therefore (with the possible exception of some tripolar spindles), the abnormalities of the division mechanism lie within the range of diversity within which the spindle must operate to give rise to regu-

lar anaphase segregation and telophase reorganization. No timing irregularities were found, and the second division was regular in all respects.

The range of structural variation was markedly increased in the hybrids, although the percentage of abnormal cells was decreased. Tripolar spindles were frequent (fig. 9), as were curved and open types (fig. 19). Even more striking were the cells with multiple spindles. The spindles in these cells were generally united with each other at the polar regions (figs. 14–16), the main spindle being either entire (fig. 14) or split into two parts (figs. 15, 16). As a rule the accessory spindles were small, always located adjacent to, or connected with, one of the major poles and possessed of either open or closed poles, or both. Only infrequently were two separate and distinct spindles formed at metaphase I (fig. 18). This figure appears similar to one in metaphase II, but the bivalents lying free in the cytoplasm show it to be otherwise.

Second division abnormalities were comparatively rare, only one (fig. 17) being found among several hundred cells.

Figure 10 is of particular interest. The spindle is somewhat curved, with the indistinct polar regions displaced to the left of the middle of the equatorial plate. The figure superficially resembles that of an unequal tripolar condition, but careful examination of this and a number of other similar metaphase configurations revealed no spindle material associated with the "third pole." Likewise the orientation of the bivalents shows no influence of a tripolar condition. Apparently the bivalents at the right side of the plate have moved into anaphase before the members situated on the left side, these still being in a metaphase state. It might be supposed that two closely approximated spindles are present, with one entering anaphase before the other; but this seems not to be the case, for even in cells with two spindles widely separated (fig. 18) the division is always exactly synchronized. In cells of this sort the spindle was always located on one side, with the equatorial plate closely abutting the cell wall. The only apparent conclusion from these anomalous metaphase-anaphase figures is that the proximity of the equatorial plate to the cell wall has upset the usual synchronization of anaphase segregation and has allowed some of the bivalents to enter anaphase before the others. If this interpretation be the true one, and the observations seem to allow of no other, it serves as evidence for BELAR'S (2) hypothesis of chromosome autonomy at the time of initial separation. If a polar force were in evidence at this time, it is difficult to envision a portion of the chromosomes moving into anaphase before the others. SAX and O'MARA (14) likewise favor chromosome autonomy, particularly where the cramped conditions of the pollen tube do not permit the functioning of a normal spindle.

Variations in the timing relationships of the spindle, in so far as they relate to the chromosome spiralization cycle, have also been found in the *piperita* × *spicata* hybrids. Development of the spindle may be precocious or delayed. When precocious, the chromosomes do not stain deeply, and the nucleolus may still be present on the metaphase plate. More often the spindle was delayed in its development. The chromosomes appear indistinctly stained, as when the spindle is precocious; but the absence of the nucleolus and the despiralized condition of the chromosomes indicate that the chromosomes are in late anaphase or early telophase, as far as their coiling is concerned (cf. figs. 11, 12 with 4). In figure

11 the spindle has failed to develop sufficiently to orient the bivalents, many of which are in a despiralized and desynapsed condition. Delayed spindles tended to be undersized (fig. 12), permitting the congression and orientation of only a limited number of bivalents on to a narrow equatorial plate lying between two masses of unoriented chromosomes.

Figure 20 presents an anomalous situation. The staining of the spindle permitted no other interpretation than that two equatorial plates were associated with but a single spindle, with the smaller upper plate lying above and to one side of the larger lower plate. Both groups of chromosomes are in early anaphase. The state of spiralization of the chromosomes indicated that the spindle was somewhat delayed. This situation is difficult to evaluate in the light of modern cell mechanics, and since it was the only cell of its kind found in this study, it seems best merely to record it until further study is made.

Discussion

This and other studies (3, 4, 6, 8) permit a more critical analysis of the origin, development, and behavior of the spindle, a structure whose function has been clearly elucidated but about which comparatively little else is known with certainty.

To understand and interpret the abnormal spindle development in *Mentha*, it is necessary to consider the origin and behavior of the normal spindle. The studies of BELAR (2), SCHRADER (16), HUGHES-SCHRADER (10), and FANKHAUSER (8) have been clarifying. They have shown that the spindle is probably a composite structure in most organisms. SCHRADER and HUGHES-SCHRADER have demonstrated in the coccids, where the centrosomes are lacking, that the spindle is a truncate, divergent affair arising from the development of the chromosome and its matrix; on the other hand, FANKHAUSER has shown that the spindle can be initiated in enucleated egg fragments, hence quite apart from the chromosomes (also the "unattached" spindles of DARLINGTON and THOMAS, 6). A normal spindle, therefore, at least of higher plants, appears to be the product of the chromosomes plus the pole determinants, co-operating with each other and synchronized in time and space, although either can produce the spindle unaided in some organisms. These are the centrosome and centromere spindles of DARLINGTON (5). The behavior of the divergent spindle (3) would seem to indicate that the centrosome portion can be dispensed with, in so far as anaphase movement is concerned, although more or less necessary for complete telophase reorganization of the nucleus.

The structural abnormalities described here seem best interpreted as variations in the behavior of the pole determinants. The centromere (chromosome?) portion of the spindle is usually present; only rarely is there complete breakdown of the spindle. Extra pole determinants, which probably arise *de novo* rather than from some invisible but permanent structure, lead to multipolar or to multiple spindles; a diffuseness at the poles, due either to the absence of a determinant or to its weak coalescent properties, leads to a divergent spindle whose structure is largely the result of chromosomal activity. OSTERHOUT (WILSON, 23) has shown in the microsporocytes of *Equisetum* that a multipolar condition regularly precedes a bipolar spindle, but it is doubtful whether such a state exists in *Mentha*.

The timing abnormalities reveal that the spindle is smaller in size the more it is delayed in time (fig. 12). This must mean that as the chromosomes begin to despiralize the centromeres are unable to

function to their full extent, and congression, orientation, and spindle formation are incomplete. This can be demonstrated in other plants. UPCOTT (21) has shown that a gene in *Lathyrus odoratus* upsets the spindle-chromosome co-ordination by delaying the spindle, while SWANSON (20) has reported that a precocious or delayed spindle can be readily induced at meiosis in *Tradescantia* by heat treatments.

Of interest from the point of view of cell mechanics are the accessory spindles (figs. 14–16). They are formed by small groups of chromosomes which had failed to be included in the major spindle. Yet apparently similar groups of free chromosomes failed to produce similar spindles (figs. 18, 19). WALD (22) has shown that the initiation of spindle formation in *Drosophila simulans* is dependent upon the presence of the II and III chromosomes. While specific chromosomes cannot be identified in *Mentha*, it is possible that the presence and activity of certain chromosomes is necessary for spindle initiation. In the case of *D. simulans*, and possibly in *Mentha*, it appears that the control of spindle-forming activity has been transferred from the individual chromosomes, as in the coccids (15), to specific chromosomes—an instance of genetic specialization.

KOLLER (11) and DARLINGTON (5) postulate polar repulsions as important in nuclear behavior. Evidence for this is largely inferential; recent data are against it. CLARK'S (3) work on divergent spindles, SCHRADER'S (18) and SAX and O'MARA'S (14) on curved spindles, DARLINGTON and THOMAS' (6) on twisted spindles, and the present study all challenge polar repulsions. Effective polar repulsions should not only bring about correct orientation of the chromosomes but should invariably produce a straight and symmetrical spindle. Such is not the case. Also equatorial orientation of the chromosomes can occur when no regular polar regions exist, implying that the agents of orientation are probably the chromosomes themselves. The autonomy of the chromosomes in initiating anaphase separation has been discussed in connection with figure 10. BELAR'S (2) theory of later anaphase movement, based on growth of the "stembody," is not supported by observations in *Mentha*, for no narrowing of the spindle in late anaphase is apparent (figs. 1, 5). CLARK (3) has shown this to be true also in maize. That the chromosomes are not alone responsible for anaphase movement is indicated by a study of cells with two spindles (fig. 18). When in anaphase, the movement of the chromosomes is always synchronized. A certain cytoplasmic condition must be necessary before the chromosomes express their autonomy in anaphase separation. The synchronous division of many adjacent cells in meiosis, both in plants and animals, bears this out.

Summary

Meiotic spindle irregularities in *Mentha piperita*, *M. spicata*, and six of their interspecific hybrids were investigated, including both timing and structural variation in spindle development and behavior. These spindle abnormalities are dependent upon the action of a gene transmitted from *M. spicata* to all its offspring. The effect of the gene is to induce an unstableness of spindle form and behavior which is subject to environmental differences. The gene exerts no directive influence on the spindle change, for irregularities were of many kinds.

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LITERATURE CITED

1. BEADLE, G. W., A gene in maize for supernumerary cell divisions following meiosis. Cornell Univ. Agr. Exp. Sta. Mem. **135**:1–12. 1931.
2. BELAR, K., Beitrage zur Kausalanalyze der Mitose. II. Untersuchungen an den Spermatocyten von *Chorthippus* (*Stenobothrus*) *lineatus* Panz. Roux's Arch. Entw. **118**:359–484. 1929.
3. CLARK, F. J., Cytogenetic studies of divergent meiotic spindle formation in *Zea Mays*. Amer. Jour. Bot. **27**:547–559. 1940.
4. CLEVELAND, L. R., Origin and development of the achromatic figure. Biol. Bull. **74**:41–55. 1940.
5. DARLINGTON, C. D., Recent advances in cytology. 2d. ed. Blakiston. 1937.
6. DARLINGTON, C. D., and THOMAS, P. T., The breakdown of cell-division in a *Festuca-Lolium* derivative. Ann. Bot. **1**:747–761. 1937.
7. DOBZHANSKY, T., Studies on hybrid sterility. I. Spermatogenesis in pure and hybrid *Drosophila psuedo-obscura*. Zeitschr. Zellf. mikr. Anat. **21**:169–223. 1934.
8. FANKHAUSER, G., Cytological studies on egg fragments of the salamander *Triton*. IV. The cleavage of egg fragments without the egg nucleus. Jour. Exp. Zool. **67**:351–391. 1934.
9. GILES, N., The effect of dehydration on microsporogenesis in *Tradescantia*. Amer. Jour. Bot. **26**:334–339. 1939.
10. HUGHES-SCHRADER, S., The meiotic chromosomes of the male *Llaveiella taenechina* Morrison (Coccidae) and the question of the tertiary split. Biol. Bull. **78**:312–337. 1940.
11. KOLLER, P. C., The movements of chromosomes within the cell and their dynamic interpretation. Genetica **16**:447–466. 1934.
12. RUTTLE, M. L., Cytological and embryological studies on the genus *Mentha*. Die Gartenbauwis. **4**:428–468. 1931.
13. SASS, J. E., Abnormal mitoses in seedlings of the Gramineae following seed treatment. Amer. Jour. Bot. **25**:624–627. 1938.
14. SAX, K., and O'MARA, J. G., Mechanism of mitosis in pollen tubes. BOT. GAZ. **102**:629–636. 1941.
15. SCHRADER, F., The chromosome cycle of *Protortonia primitiva* and a consideration of the meiotic division apparatus in the male. Zeitschr. wiss. Zool. **138**:386–408. 1931.
16. ———, Recent hypotheses on the structure of spindles in the light of certain observations in Hemiptera. Zeitschr. wiss. Zool. **142**:520–539. 1932.
17. ———, The present status of mitosis. Amer. Nat. **74**:25–33. 1940.
18. ———, Chromatin bridges and irregularity of mitotic coordination in the pentatomid *Peromatus notatus*. Amer. Serv. Biol. Bull. **81**:149–161. 1941.
19. SCHURHOFF, P. N., Cytologische und genetische Untersuchungen an *Mentha* und ihre Bedeutung für die Pharmacognosie. Arch. Pharmaz. Ber. deutsch. Pharmaz. Ges. **267**:515–526. 1929.
20. SWANSON, C. P., Meiotic coiling in *Tradescantia*. BOT. GAZ. **103**:457–474. 1942.
21. UPCOTT, M. B., Timing unbalance at meiosis in the pollen-sterile *Lathyrus odoratus*. Cytologia Fujii Jub. Vol. 299–310. 1937.
22. WALD, H., Cytologic studies on the abnormal development of the eggs of the *claret* mutant type of *Drosophila simulans*. Genetics **21**:264–281. 1936.
23. WILSON, E. B., The cell in development and heredity. 3d ed. Macmillan. 1925.

COMPARATIVE ACTIVITY OF SYNTHETIC AUXINS AND DERIVATIVES

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Introduction

The object of the present study was to determine the comparative activity of both known and new synthetic auxins and derivatives, applied to intact and decapitated *Avena* test plants in two carriers, agar and lanolin. The technique which employs the *Avena* coleoptile is considered by many a preferred method for the quantitative determination of auxin activity. KÖGL and KOSTERMANS (7) with this method, and KOEPFLI (6) with the pea test, found that compounds which promote growth must, among other requirements, possess a free carboxyl group, or—if esterified—be readily hydrolyzable; their tests all involved the use of agar or aqueous solution as a carrier.

When the bending response of tomato leaves and stems was used as a criterion of auxin activity, using lanolin as a carrier, ZIMMERMAN and HITCHCOCK (12, 13) found that the methyl esters of indoleacetic and indolebutyric acids were more than twice as active as the free acids; methyl naphthaleneacetate was half as active as naphthaleneacetic acid.

METHODS.—In the course of this work, various synthetic auxins, and the amides and esters of some of them, have been investigated by the deseeded test method of SKOOG (see 1). To determine the influence of the carrier on the results obtained, lanolin as well as the usual agar blocks was applied to decapitated coleoptiles. Such lanolin blocks were prepared by pouring warm lanolin containing the various substances, in different concentrations, into standard-volume brass molds standing directly upon ice cakes. The blocks cut from these were of the same volume as the agar ones (10 mm.^{3}) and were applied to decapitated coleoptiles in the same manner that the agar blocks were applied.

In tests involving the use of lanolin on intact coleoptiles, the auxins and their derivatives were mixed in anhydrous lanolin and applied unilaterally to the distal 5 mm. of intact *Avena* coleoptiles (on the narrow side of the coleoptile, parallel with the underlying vascular bundle). In those experiments where agar blocks were applied to the side of intact coleoptiles, they were made to adhere by first applying a small drop of 5 per cent gelatin.

Avena plants (variety Victory) were grown in the usual water cultures. All were deseeded, and the same conditions of temperature and humidity (25° C. and 85–90 per cent) were maintained during the application period for both lanolin and agar tests. Conclusions as to activity were based on shadowgraphs of test plants taken after 5 hours.

Experimental results

APPLICATION IN AGAR TO DECAPITATED COLEOPTILES

Potassium salts of indoleacetic, indolebutyric, and naphthaleneacetic acids are as active as free acids (table 1). This is in accord with previous results ob-

tained by the *Avena* assay (4, 11, 2) and by the tomato-bending response (13).

TABLE 1

RELATIVE ACTIVITY OF SYNTHETIC AUXINS, DETERMINED BY DESEEDED AVENA TECHNIQUE, USING AGAR AS THE CARRIER (ON DECAPITATED COLEOPTILES). AT LEAST 12 TEST PLANTS WERE USED PER DILUTION ASSAYED, AND ALL CURVATURES ARE IN THE PROPORTIONALITY RANGE. IN MOST INSTANCES, CALCULATIONS OF RELATIVE ACTIVITY IN COMPARISON WITH INDOLEACETIC ACID ARE BASED ON CURVATURES OBTAINED WITH THE LOWER CONCENTRATIONS

COMPOUND	SOURCE*	CONCENTRATION (MG.) PER LITER OF AGAR MIXTURE; AVENA CURVATURE IN DEGREES				RELATIVE EFFECTIVENESS (INDOLEACETIC ACID = 100)
		CONC.	CURV.	CONC.	CURV.	
Indoleacetic acid	VIII	0.010	10.7‡	0.020	19.6‡	100
Potassium indoleacetate	III	0.010	10.1	0.020	21.5	94
Methyl indoleacetate	II	0.010	6.6	0.020	12.6‡	62
	III	0.010	6.5†	0.020	13.8†	61
Ethyl indoleacetate	II	0.020	6.2‡	0.040	10.5†	29
	IX	0.020	6.0†	0.040	10.8†	28
Indoleacetamide§	IV	0.500	0	2.0	0	0
	V	0.500	0	1.0	3.7†	0.4
Indolebutyric acid	III	0.100	4.1	0.200	8.7	4
	VIII	0.160	8.3	0.320	17.0	5
Potassium indolebutyrate	III	0.200	8.1†	0.400	16.5	4
Methyl indolebutyrate	I	0.160	8.1	0.320	17.5	5
	II	0.160	8.0	0.080	4.9	5
	III	0.160	6.9†	0.320	14.1	4
Ethyl indolebutyrate	II	0.160	8.1	0.320	12.0†	5
Naphthaleneacetic acid (all derivatives are alpha form)	IV	0.050	10.1	0.100	19.8	19
	III	0.050	10.3	0.100	18.7†	19
Potassium naphthaleneacetate	III	0.050	8.6	0.100	18.8	16
Methyl naphthaleneacetate	II	2.56	2.8	5.12	4.7	0.09
	III	2.56	2.1†	5.12	3.3†	0.06
	VI	2.56	0†	5.12	1.7†	0.03
Ethyl naphthaleneacetate	I	2.56	0	5.12	0	0
Naphthaleneacetamide§	IV	2.5	0†	5.0	0	0
	VI	2.56	0†	5.12	2.9	0.05
	VII	2.56	0	5.12	4.8	0.09
Naphthoxyacetic acid (all derivatives are beta form)	IX	0.2	0	5.0	0	0
Ethyl naphthoxyacetate	IX	0.2	0	5.0	0	0
Naphthoxypropionic acid	IX	0.2	0	5.0	0	0
Dichlorophenoxyacetic acid	IV	5.0	6.7	10.0	10	0.1

* Source of compounds: I–III, from P. W. Zimmerman, Boyce Thompson Institute for Plant Research (I received 1936; II and III received in February and July, 1941). IV, Dow Chemical Company. V, K. V. Thimann, Harvard University. VI, American Chemical Paint Company. VII, R. H. Manske, National Research Council of Canada. VIII, Merck and Company. IX, P. W. Zimmerman (received March, 1942).

† Average of 24 test plants.

‡ Average of 36–60 test plants.

§ Upon hydrolysis with 1N NaOH, indoleacetamide and naphthaleneacetamide gave solutions which, after neutralizing, had at least half the auxin activity which could be expected from a theoretical yield of free acids.

In general, the esters are less active than the corresponding free acids, with the exception of the methyl and ethyl esters of indolebutyric acid; these appear to be as active as the free acid. Ethyl indoleacetate is only one-third as active as the free acid; and the methyl and ethyl esters of naphthaleneacetic acid are almost completely inactive. These results are in general similar to those re-

ported elsewhere for ester derivatives; that is, esters of certain active acids are active, while others are inactive. Indoleacetamide and naphthaleneacetamide are almost completely inactive. β-naphthoxyacetic acid, its ethyl ester, and β-naphthoxypropionic acid are also completely inactive.

The response of *Avena* to esters of naphthaleneacetic and naphthoxyacetic acids, and the amides of indoleacetic and naphthaleneacetic acids, has not been reported on heretofore. Dichlorophenoxyacetic acid, recently shown to be active in inducing parthenocarpy and leaf-form changes (15), shows but slight activity in the *Avena* test.

Application in agar to intact coleoptiles

No curvatures were obtained with indoleacetic, indolebutyric, and naphthaleneacetic acids when applied in agar to intact coleoptiles. All were tested at concentrations of 0.5, 1.0, and 5.0 mg. per liter; in addition, indoleacetic acid was tested at 10, 20, and 40 μgm. per liter.

Laibach and Kornmann (8) applied agar blocks containing auxin from pollen of several different species of orchids at different levels on intact *Avena* test coleoptiles. Those applied near the tip gave very small curvatures, whereas those applied below the tip—at one-third to one-half the length of the coleoptile—induced pronounced curvatures within 5 hours. Following their technique as closely as possible, and using the preceding concentrations of the synthetic substances, no *Avena* curvatures were obtained. Extracts of hickory pollen, which by assay on decapitated coleoptiles give 4.6–9.6 million TDC per gram (equivalent to 46–96 μgm. indoleacetic acid per gram), also give no curvatures when applied in agar blocks to intact coleoptiles. The concentration used (1:100) approximates that employed by Laibach and Kornmann.

Application in lanolin blocks to decapitated coleoptiles

When lanolin is the carrier (table 2), concentrations of the acids required to produce *Avena* curvatures close to 10° are approximately ten to fifty times as great as for agar. The activity ratios of indoleacetic, indolebutyric, and naphthaleneacetic acids, when applied in lanolin, are 100:11:11, respectively; whereas in agar the ratios are 100:5:19. The response to indoleacetic acid in each of the two carriers is arbitrarily set at 100.

TABLE 2

Relative activity of synthetic auxins as determined by deseeded Avena technique, but employing blocks of lanolin rather than of agar

Compound	No. of test plants	Concentration per liter of lanolin (mg.)	Avena curvature (°)	Relative effectiveness (indoleacetic acid = 100)
Indoleacetic acid	24	0.3*	8.0	100
Indoleacetic acid	24	0.6	11.9	.....
Indolebutyric acid	24	2.5*	7.5	11
Indolebutyric acid	24	5.0	7.5	.....
Naphthaleneacetic acid	12	2.5*	7.4	11
Naphthaleneacetic acid	24	5.0	11.2	.....

* Still lower concentrations give curvatures too inconsistent to be relied upon; these may therefore be considered threshold values.

The lack of proportionality curvatures (table 2) is to be noted.

Application in lanolin to intact coleoptiles

The data from lanolin tests on whole coleoptiles are presented in table 3. The

comparative activities of the several derivatives in relation to their corresponding acids are not greatly different, whether applied in agar or in lanolin. Methyl and ethyl indoleacetates are the outstanding exceptions: the former is one and one-half times as active as the acid, the latter only about one-twentieth as active; the ethyl ester of indolebutyric is only one-half as active as the free acid.

Indoleacetamide, when applied in lanolin to intact coleoptiles, gives a threshold response at 1500 mg. per liter of lanolin, only one five-thousandths as active as the free acid. Naphthaleneacetamide does not give any response when tested at concentrations ranging from 5 to 5000 mg. per liter of lanolin. Hence, in the *Avena* test the amides are essentially inactive, whether applied in agar or in lanolin, to decapitated or intact coleoptiles.

Threshold concentrations in lanolin are the same, whether the coleoptiles are intact or decapitated, in spite of the fact that the area of application to the test plants differs.

TABLE 3

RELATIVE ACTIVITY OF SYNTHETIC AUXINS, DETERMINED BY UNILATERAL APPLICATION IN ANHYDROUS LANOLIN TO WHOLE AVENA COLEOPTILES (DESEEDED PLANTS). AT LEAST 12 TEST PLANTS USED PER DILUTION ASSAYED. CURVATURES REPRESENT THRESHOLD RESPONSES (LOWEST CONCENTRATIONS GIVING CURVATURES OF 5–10 DEGREES)

COMPOUND	SOURCE*	CONCENTRATION PER LITER LANOLIN REQUIRED TO GIVE THRESHOLD RESPONSE (MG.)	CURVATURES (°)	RELATIVE EFFECTIVENESS (INDOLEACETIC ACID = 100)
Indoleacetic acid	VIII	0.3	9.0	100
Methyl indoleacetate	II	0.2	9.1	150
Ethyl indoleacetate	II	5.0†	6.6	6
Indoleacetamide	IV	1500.0	14.8	0.03
Indolebutyric acid	VIII	2.5	6.6	12
Methyl indolebutyrate	II	2.5	5.6	12
Ethyl indolebutyrate	II	5.0	6.8	6
Naphthaleneacetic acid	IV	2.5	6.7	12
Methyl naphthaleneacetate	VI	No response‡	0	0
Naphthaleneacetamide	IV	No response§	0	0
Naphthoxyacetic acid	IX	No response‖	0	0
Ethyl naphthoxyacetate	IX	No response‖	0	0
Naphthoxypropionic acid	IX	No response‖	0	0
Dichlorophenoxyacetic acid	IV	80.0	3.8	0.2

* Sources as for table 1.

† Gave very inconsistent results in four sets of tests on three batches of materials. Value reported is probably maximum.

‡ No curvature in six sets of tests at concentrations of 5–80 mg. per liter of lanolin.

§ No curvature in three sets of tests at 5–160 mg. per liter of lanolin.

‖ No curvature in two sets of tests at 2.5–10 mg. per liter of lanolin.

LEVULINIC ACID

Reports finding their way into the trade literature (5) regarding the activity of levulinic acid make it of interest to assay this substance in the usual *Avena* test. No activity is obtained at concentrations of 0.1, 0.2, 1.0, and 5.0 mg. per liter of agar mixture. Negative results are obtained also when it is tested in lanolin on intact coleoptiles at concentrations of 2.5, 5.0, and 10.0 mg. per liter of

lanolin. To determine whether it might possibly increase the activity of an auxin such as indoleacetic acid, levulinic acid in concentrations of 0.2 and 1.0 mg. per liter was made up in 1.5 per cent agar with indoleacetic acid (the latter at a 0.010 mg. per liter concentration). The lower concentration of levulinic acid, with the indoleacetic, gave *Avena* curvatures of 10.7°; the higher concentration, 11.0°. Indoleacetic-acid controls tested at the same time gave curvatures of 11.7°. Earlier tests on levulinic acid in straight growth of the *Avena* coleoptile (2) were also negative. Thus this compound induces no response in the *Avena* test, nor does its presence accelerate the response of the coleoptile to indoleacetic acid.

Discussion

It has been found that (*a*) when applied in agar to decapitated *Avena* plants, certain auxins as free acids are more active than ester or amide derivatives, which agrees with and extends data already in the literature; (*b*) when applied in lanolin to whole (intact) *Avena* coleoptiles, one ester is more active than the corresponding free acid, which only partly confirms data in the literature (in which tomato was used as the test plant). The hypothesis that the ester and amide derivatives must first be hydrolyzed before they are active still cannot be answered directly. In agar, both the methyl and ethyl esters of indolebutyric acid are apparently hydrolyzed readily; with indoleacetic acid, only the methyl ester; with naphthaleneacetic acid, apparently neither ester is hydrolyzed. In lanolin the exceptional activity of methyl indoleacetate might be interpreted as indicating that hydrolysis need not occur; there is insufficient evidence to eliminate other possibilities.

The data indicate that when the same test plant is used (*Avena*), the relative activities of auxins differ according to the nature of the carrier in which they are applied. Ten to fifty times the concentration of different growth substances is required for threshold responses when applied in lanolin (whether blocks or unilateral smears) as compared with agar, indicating that the rate of penetration of auxins from lanolin into decapitated *Avena* test coleoptiles is markedly slower than that from agar. Against this is the fact that the substances applied in agar to intact coleoptiles induced no response at all. This latter point can only mean that when the epidermis is intact, synthetic growth substances in agar penetrate into the underlying tissue so slowly that no response is induced in the 5-hour test period. The extent to which the agar makes a poor contact with the coleoptile tissue is not known, and this also may be an important factor in the failure of agar as a carrier on intact coleoptiles. LAIBACH and KORNMANN'S positive results were obtained with orchid pollen extracts. SNOW (10) found that extracts of pea shoots gave both positive and negative curvatures, when applied in strips of blotting paper to intact *Avena* coleoptiles.

MITCHELL and STEWART (9) report that naphthaleneacetamide is inactive in agar, in the *Avena* test (0.25 mg. per liter of agar), and also when unilaterally applied to stems of tomato plants at 2 per cent in lanolin. However, they obtained *Avena* curvatures when naphthaleneacetamide was applied unilaterally at 172 mg. per liter of lanolin (0.02 per cent).

Different species differ in degree of response to a given compound in a given carrier; tomatoes and *Avena*, for example, do not have the same threshold re-

sponses to different synthetic auxins applied in lanolin (table 4). Such data are not strictly comparable, because the tomatoes were greenhouse grown, and temperatures doubtless fluctuated considerably. It is clear, however, that for the active compounds it requires eight to eighty times the concentration in lanolin to induce the tomato response as compared with the *Avena* response. Certain compounds, such as naphthoxyacetic acid and methyl naphthaleneacetate, are completely inactive on *Avena* but are very active in inducing epinasty and other responses in tomato (12, 14).

It would seem that, given the same carrier (lanolin), the response of different test plants depends in part upon their respective growth rates and in part upon their species. In the case of *Avena* vs. tomato, it is reasonable to expect that etiolated coleoptiles of *Avena* grow more rapidly than greenhouse-grown tomato plants; hence the necessity of higher concentrations of growth substances to induce the response in tomato. However, some compounds are active on tomato which are completely inactive on *Avena*. Given a different carrier (agar vs. lanolin) and the same kind of test plant (such as *Avena*), the response depends upon the rate of penetration from the carrier.

TABLE 4

RELATIVE ACTIVITY OF SYNTHETIC AUXINS, DETERMINED BY UNILATERAL APPLICATION IN ANHYDROUS LANOLIN TO WHOLE AVENA COLEOPTILES AND TOMATO PETIOLES (ZIMMERMAN). AT LEAST 12 TEST PLANTS USED PER DILUTION ASSAYED. ONE LITER OF LANOLIN = 860 GM.

COMPOUND	SOURCE	THRESHOLD CONCENTRATION REQUIRED TO GIVE DEFINITE CURVATURES AFTER 5 HOURS, MG./L. OF LANOLIN (FROM TABLE 3)	THRESHOLD VALUES, % IN LANOLIN		
			AVENA COLEOPTILE (FROM TABLE 3)	TOMATO PETIOLE (ZIMMERMAN ET AL.)	
				1936	1937
Indoleacetic acid	VIII	0.3	0.000035	0.00125	0.0001
Methyl indoleacetate	II	0.2	0.000023	0.0006	0.0001*
Ethyl indoleacetate	II	5.0†	0.00058	.....	0.0001*
Indoleacetamide	IV	1500	0.17	.....	No response*
Indolebutyric acid	VIII	2.5	0.00029	0.025	0.01
Methyl indolebutyrate	II	2.5	0.00029	0.01	.....
Ethyl indolebutyrate	II	5	0.00058	.....	.....
Naphthaleneacetic acid	IV	2.5	0.00029	0.0025	0.0001
Methyl naphthaleneacetate	VI	No response	.....	0.005	0.0001*
Naphthaleneacetamide	IV	No response	.....	.....	ca. 0.0001*
Naphthoxyacetic acid	IV	No response	.....	.....	0.005*

* Private communication. We are grateful to Dr. P. W. Zimmerman for certain samples, and for permission to include these data.
† After 10 hours there sometimes are threshold curvatures at 5 mg./l., but such results are irregular.

The preceding data emphasize still further (*a*) the conclusion of several investigators (11, 13, 3) that no one test plant or test method can suitably serve for determining the relative activities of various synthetic auxins, and (*b*) the importance of the carrier in determining relative activities, even when the same test plant is used.

Summary

The activity of indoleacetic, indolebutyric, naphthaleneacetic, dichlorophenoxyacetic, and naphthoxyacetic acids

and certain derivatives has been tested on deseeded *Avena* coleoptiles. Intact as well as the usual decapitated coleoptiles were employed, and both agar and lanolin blocks (and lanolin in unilateral smears) were used as carriers.

1. When tested in agar on decapitated coleoptiles, potassium salts of indoleacetic, indolebutyric, and naphthaleneacetic are as active as the free acids. Esters of indolebutyric are of about the same activity as the acid; esters of indoleacetic are less active than the corresponding acid; and esters of naphthaleneacetic acid are inactive. Indoleacetamide, naphthaleneacetamide, and dichlorophenoxyacetic acid are but slightly active. Naphthoxyacetic, its ethyl ester, and naphthoxypropionic acids are completely inactive (although reported by others to be active in the tomato test).

2. When applied in agar to intact coleoptiles, indoleacetic, indolebutyric, and naphthaleneacetic acids are completely inactive.

3. When blocks of lanolin rather than agar are applied to decapitated coleoptiles, ten to fifty times the concentration of indoleacetic, indolebutyric, and naphthaleneacetic acids must be used to give threshold curvatures. In agar, the relative activities of the acids are 100:5:19, respectively; in lanolin, 100:11:11.

4. When applied in lanolin to intact coleoptiles, indoleacetic, indolebutyric, and naphthaleneacetic acids have the same activity as when applied to decapitated coleoptiles; that is, threshold concentrations are the same whether the coleoptiles are intact or decapitated.

5. Levulinic acid is completely inactive in the *Avena* test, whether applied in lanolin or agar, and has no complementary effect when applied together with indoleacetic acid.

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LITERATURE CITED

1. AVERY, G. S., JR., CREIGHTON, H. B., and HOCK, C. W., A low cost chamber for phytohormone tests. Amer. Jour. Bot. **26**:360–365. 1939.
2. AVERY, G. S., JR., and SARGENT, G. B., The effect of various compounds upon straight growth of the *Avena* coleoptile. Amer. Jour. Bot. **26**:548–555. 1939.
3. AVERY, G. S., JR., CREIGHTON, H. B., and SHALUCHA, B., Expression of hormone yields in relation to different *Avena* test methods. Amer. Jour. Bot. **28**:498–506. 1941.
4. BONNER, D. M., Activity of the potassium salt of indole-3-acetic acid in the *Avena* test. BOT. GAZ. **99**:408–411. 1937.
5. IRELAND, J. C., New miracles with field crops performed. Southern Seedsman **5**:9; 36. 1942.
6. KOEPFLI, J. B., THIMANN, K. V., and WENT, F. W., Phytohormones: structure and physiological activity. Jour. Biol. Chem. **122**:763–780. 1938.
7. KÖGL, F., and KOSTERMANS, D. G. F. R., Über die Konstitutions-Spezifität des Hetero-auxins. Hoppe-Seyl. Zeitschr. Phys. Chem. **235**:201–216. XVI. Mitteilung. 1935.
8. LAIBACH, F., and KORNMANN, P., Zur Frage des Wuchsstofftransportes in der Haferkoleoptile. Planta **21**:396–418. 1933.
9. MITCHELL, J. W., and STEWART, W. S., Comparison of growth responses induced in plants by naphthalene acetamide and naphthalene acetic acid. BOT. GAZ. **101**:410–427. 1939.
10. SNOW, R., An inhibitor of growth extracted from pea leaves. Nature (London) **144**:906. 1939.
11. THIMANN, K. V., and SCHNEIDER, C. L., The relative activities of different auxins. Amer. Jour. Bot. **26**:328–333. 1939.
12. ZIMMERMAN, P. W., HITCHCOCK, A. E., and WILCOXON, F., Several esters as plant hormones. Contrib. Boyce Thompson Inst. **8**:105–112. 1936.
13. ZIMMERMAN, P. W., and HITCHCOCK, A. E., Comparative effectiveness of acids, esters, and salts as growth substances and methods of evaluating them. Contrib. Boyce Thompson Inst. **8**:337–350. 1937.
14. ———, and ———, Formative effects induced with beta-naphthoxyacetic acid. Contrib. Boyce Thompson Inst. **12**:1–14. 1941.
15. ———, and ———, Substituted phenoxy and benzoic acid growth substances and the relation of structure to physiological activity. Contrib. Boyce Thompson Inst. **12**:321–343. 1942.

ANATOMY AND HISTOLOGY OF THE EUREKA LEMON

ERNEST S. FORD

(WITH FORTY-SEVEN FIGURES)

Introduction

VOLKAMER (62) and RISSO and POITEAU (43) described and illustrated gross aspects of many species of *Citrus*. Their reports are of morphological significance mainly because they served as the background for the work of BONAVIA (9), who, without significant anatomical data of his own, presented an interpretation of the citrus fruit based upon comparative anatomy. He regarded the rind as one whorl of sterile carpels, while the pulp was considered a second whorl of carpels which were fertile and whose oil glands (juice sacs) protruded into the locules. Evidence for this concept of the juice sacs was found in the related genus *Oegle*, where more definite oil glands were found projecting from the pericarp into the locules. BONAVIA'S concept of the two whorls of carpels is similar to SAUNDERS' (45) concept of the nature of the angiosperm gynoecium.

Phases of the macroscopic structure of the fruits of several species of *Citrus* have been described (11, 12, 25, 51, 65). ROSS (44), in describing the lemon fruit, applied the classic concept of the carpel in interpreting the different vascular bundles of the gynoecium, although he did not follow their courses. He referred to the juice sacs as carpel hairs but presented no more histological data than had GREW (25) three centuries earlier.

References to teratological flowers and fruits of *Citrus* have been discussed by PENZIG (39) and MASTERS (32). Among these are the instances of extra whorls of carpels and the occurrence of sterile digitate carpels. The latter, in a variety of lemon, has been used by SAUNDERS (46) as evidence in favor of the theory of polymorphic carpels. In this variety only the dorsal part of each carpel elongates, while the marginal portion remains unseparated from the apex of the receptacle. Neither ovules nor locules are produced. This was interpreted as failure of the "fertile carpels" to develop while the "sterile carpels" do develop and form the anomolous structures.

PAYER (37) has presented the ontogeny of the floral parts of *C. aurantium*. PENZIG (38) has discussed histological data for the same species. He disagreed with PAYER and considered two whorls of stamens present rather than one. Also, his account of the courses of vascular bundles differs from that of VAN TIEGHEM (61) for the same species. Some histological data on *C. decumana* and *C. hystrix* were given by comparison. TSCHIRCH (58) described and figured some aspects of histology of the gynoecium and fruit of *C. vulgaris*. Both he and PENZIG (38) distinguished two kinds of carpellary emergences into the locule. The long ones, the juice sacs, were considered histologically different from, though similar in origin to, the short dome-shaped ones which had papillose cells and which TSCHIRCH thought secreted mucilaginous material into the locule. He described the formation of the oil glands. The structure of the rind has been discussed also by others

(8, 9, 14, 19, 20, 29, 38, 40) for various species.

BIERMAN (8) presented data on the cytoplasmic inclusions of the parenchymatous cells of the fruit of *C. vulgaris* and described seeds with several embryos in this and other species. OSAWA (35) dealt mainly with sporogenesis and embryogeny in *C. trifoliata* and gave some causes of the seedless condition in Washington Navel orange and in *C. nobilis*. Parthenocarpy (26), fertilization (13), and nucellar embryos (22, 23) have also been discussed. Recently data on blooming and fruit setting have been reported for several species (1, 42).

Several reports (33, 50, 51, 56) have appeared on the taxonomy and phylogeny of that section of the Rutaceae to which *Citrus* belongs. One of these (56) attempts to correlate the degree of fusion of the vascular traces, mainly those to perianth and stamens, with phyletic groups within the family and to make clear the nature of the stylar canals, interpreting the gynoecial disk as a vestigial stamen whorl. In some species definite correlation between location of oil glands and ends of bundles was found.

Material and methods

Collections were made of flowers and fruits of Eureka lemon of various ages at Lower Rio Grande Valley Experiment Station, Westlaco, Texas, by S. H. YARNELL in 1938 and by J. F. WOOD in 1941 and 1942. Certain stages were collected also at Riverside, California, by Dr. H. E. HAYWARD in 1941. The material was killed in various solutions, and serial sections were prepared by the paraffin technique. Young inflorescenses, flowers, and fruits up to 15 mm. in diameter were imbedded and sectioned whole, while only suitable pieces of larger fruits were used. Considerable difficulty arose from the presence of quantities of hesperidin raphides throughout the inner parts of fruits of certain ages. No satisfactory way of removing them was found.

Observations

PEDICEL

Cymose flowers arise in axils of the leaves. Pedicels are very short and are subtended by single minute bracts. Many of the lateral flowers contain well-developed stamens but abortive pistils. These and other flowers which do not set fruit abscise at the base of the pedicel, although the petals, stamens, and pistils sometimes abscise first.

The cortex is composed of isodiametric cells and abundant air spaces. Oil glands occur frequently in the outer part of the cortex. Like those in the fruit, they begin differentiation early in the ontogeny of the organ, and new ones are formed during its maturation.

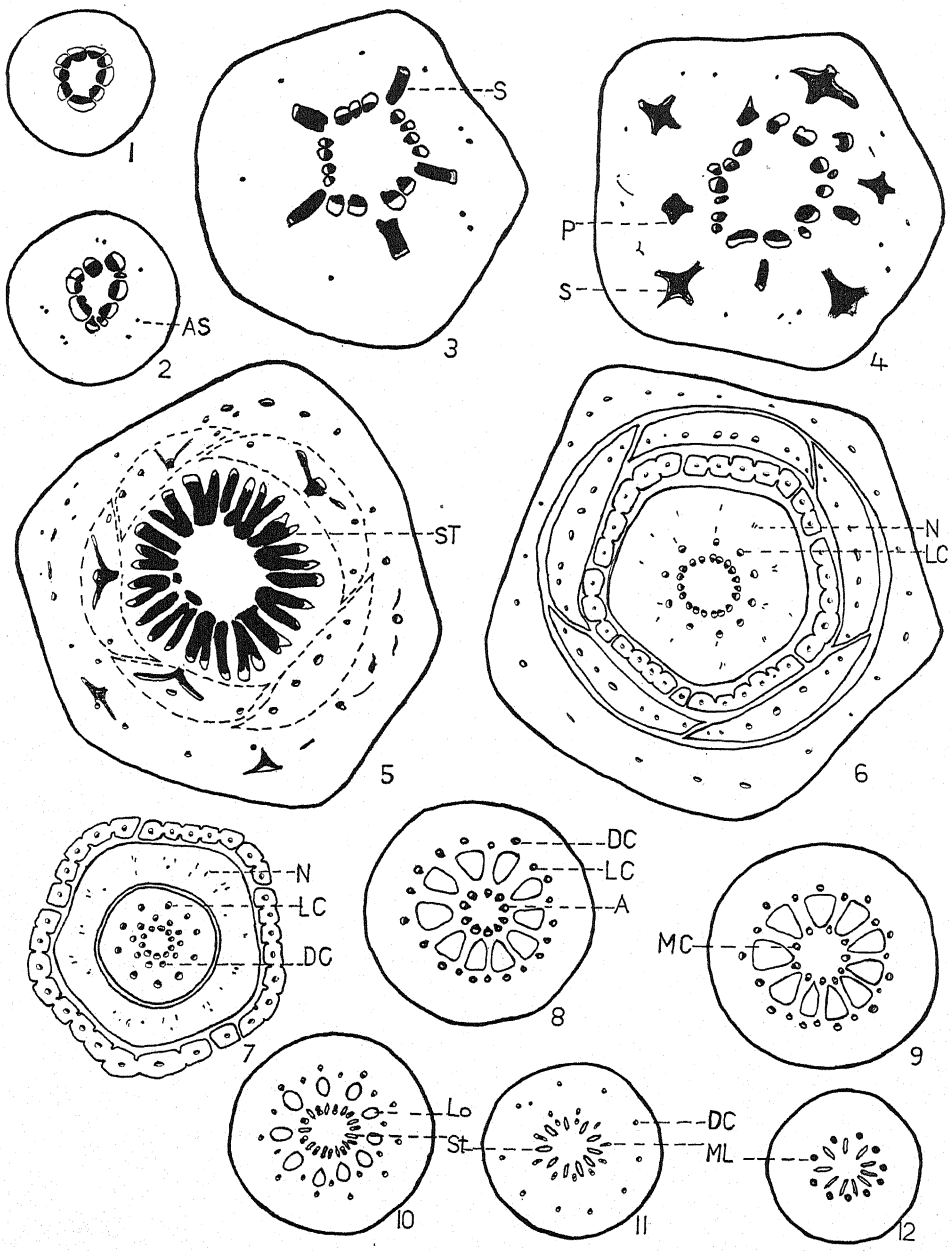
An endodermis is not well differentiated, but the transition to small cells of the pericycle usually is abrupt. The pericycle in pedicels of flowers is entirely composed of thin-walled cells, but in older stages small groups of strongly lignified cells occur adjacent to the phloem.

Some five to nine collateral bundles make up the vascular cylinder (fig. 1). Cambial activity is largely fascicular but finally may become interfascicular as well. Secondary thickening is not great, and most of the elements produced are not lignified, even when the fruit is half mature.

The pith usually does not exceed the width of the cortex. Its cells are isodiametric, and intercellular spaces are small. Many cells of the pith are lignified when the fruit is mature.

RECEPTACLE

The transition from pedicel to receptacle is gradual. The latter is consider-



FIGS. 1–12.—Figs. 1–4, cross-sections of pedicel and receptacle showing arrangement of perianth traces. *as*, accessory sepal traces; *s*, sepal trace; *p*, petal trace. Fig. 5, section showing divergence of stamen traces (*st*). Figs. 6, 7, sections at level of base of ovary showing order of divergence of nectary bundles and two lowest carpel traces. *lc*, lateral carpel (septal) traces; *dc*, dorsal carpel traces. Fig. 8, section of ovary below divergence of marginal carpel bundles. *a*, axial or receptacular bundle. Fig. 9, section of ovary above end of residual vascular tissue showing inverted marginal carpel bundles (*mc*). Fig. 10, section above placenta showing position of stylar canals (*st*) and tops of locules (*lo*). Fig. 11, section through base of style showing convergence of marginal and lateral carpel bundles (*ml*) and position of dorsal carpel bundles (*dc*). Fig. 12, section through lower part of stigma.

ably larger in diameter than the pedicel, because the cells of the cortex and pith are larger and more numerous. There are two series of sepal traces. Below the bases of the sepals are several groups of small vascular traces. Some of these are not attached below, but others diverge from the vascular cylinder about 3 mm. below the bases of the sepals. All these bundles, accessory sepal traces (fig. 2), extend into the lateral part of the sepals. Main sepal traces diverge from the vascular cylinder at a level of bases of the sepals. In flowers with five sepals there are five main sepal traces (fig. 3), each of which has three branches. The median one of these branches is connected with the median bundles of a sepal, while the other two are curved upward and are joined with the lateral bundles of the adjacent petals.

Receptacular bundles are curved in such a way that the gaps above the main sepal traces are closed, and a whorl of petal traces diverges from the vascular cylinder at this level. These traces are equal in number to the sepal traces and alternate with them. Each petal trace has three branches (figs. 4, 5). The median one is connected with the petal bundles, while the two lateral ones extend into the marginal portions of two adjacent sepals. There is considerable anastomosis and branching of the lateral branches of the sepal and petal traces beneath the bases of sepals and petals.

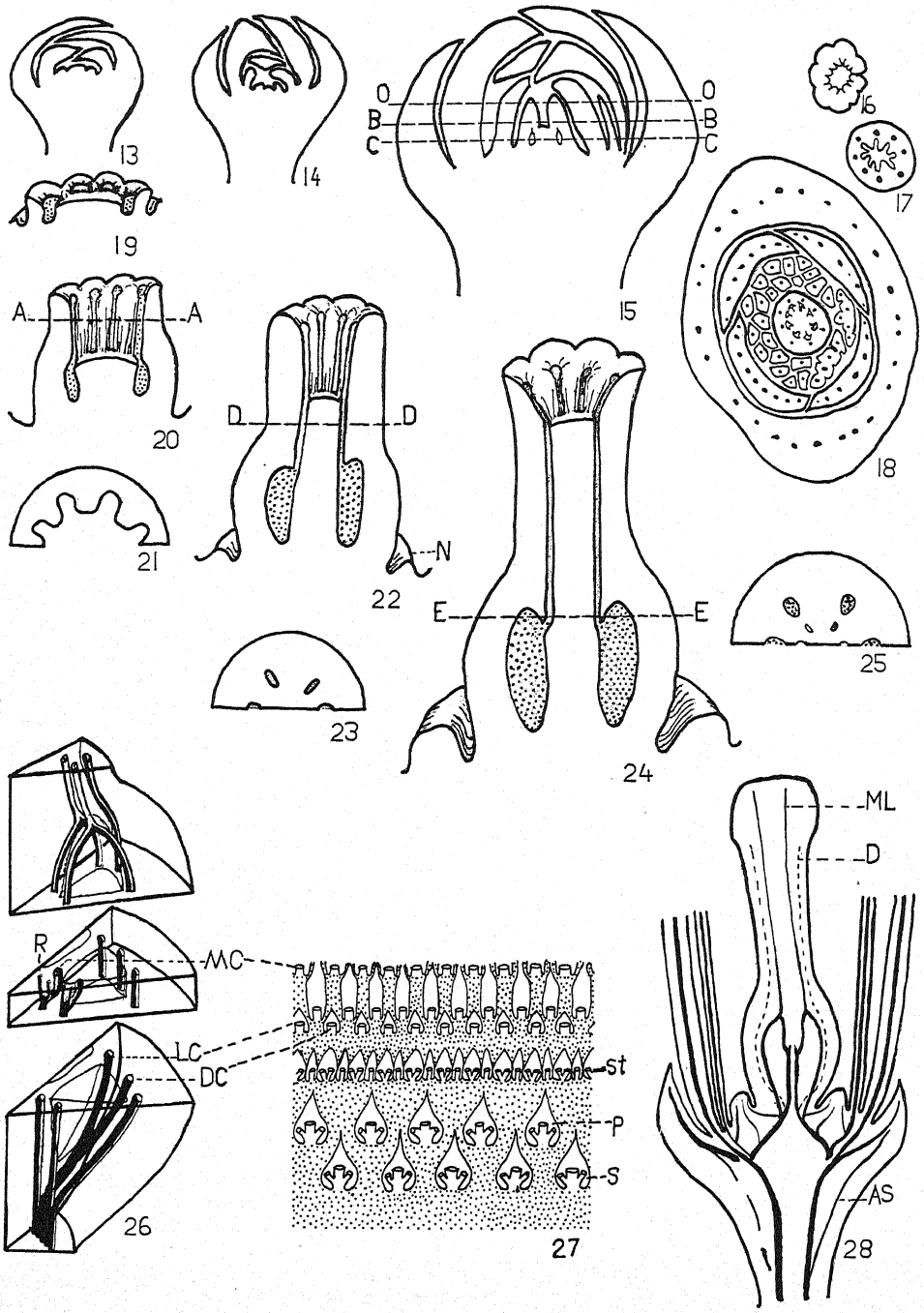
A whorl of fifteen to twenty stamen traces (fig. 5) diverges from the bundles of the axis above the level of the gaps of the petals. Approximately ten of these traces are forked immediately, so that there are about twenty-five to thirty bundles which extend into an equal number of stamens. In flowers which have five sepals usually the traces of the episepalous stamen are unbranched, but they appear at the same level as other stamen traces, not below them, as PENZIG (38) reported for orange.

Theoretically each carpel has one dorsal bundle, two lateral or septal bundles (which are usually undiverged from those of adjacent carpels), and two similarly undiverged marginal bundles. The lateral carpel bundles diverge from the vascular cylinder just above the level of the gaps of the stamens (fig. 6). There is no specific location of these bundles in alternation with those of other floral parts below, because of the variable number of stamens and carpels.

The dorsal carpel bundles diverge from the vascular cylinder just above, and alternate with, the gaps above the lateral bundles (fig. 7). But the gaps above the dorsal carpel bundles extend several millimeters, to the level of the center of the ovary (figs. 26, 27). At this level the marginal carpel bundles diverge into positions alternate with the dorsal ones, directly inward from the septa (figs. 9, 26). The receptacular bundles terminate at the level of the divergence of the marginal carpel bundles or extend slightly above and close some of the gaps above the dorsal bundles (fig. 27).

CALYX

The primordia of the three to six sepals are first to appear and originate separately in a whorl, although almost immediately meristematic activity begins between the primordia, and growth of the whole zone produces the synsepalous portion of the calyx which arches over the apex of the receptacle until the developing petals force it aside (figs. 13, 14). The calyx usually is less than 1 cm. long, but its base becomes thick and broadened slightly into the saucer-shaped structure which persists when the fruit is mature.



FIGS. 13–28.—Figs. 13, 14, longisections of flower bud showing origin of stamen and carpel primordia, respectively. Fig. 15, longisection of older flower bud prior to formation of gynoecial disk. Figs. 16, 17, cross-sections of carpels at *OO* and *BB*, respectively. Fig. 18, cross-section of flower bud at *CC*. Figs. 19–25, diagrams of successive stages in development of carpels showing ontogeny of stylar canals and nectary. Fig. 21, cross-section at *AA;* fig. 23, at *DD;* fig. 25, at *EE*. Fig. 26, diagram of sector of young fruit showing arrangement of main vascular bundles of one locule. *r*, receptacular residual tissue; *mc*, *lc*, *dc*, marginal, lateral, and dorsal carpel bundles. Fig. 27, diagram representing vascular axis of flower slit lengthwise and laid out flat; position of various traces somewhat idealized. *st*, stamen traces; *p*, petal trace; *s*, sepal trace. Fig. 28, longisection of flower diagrammatically showing relation of vascular bundles. *ml*, extension of both marginal and lateral carpel bundles; *d*, dorsal carpel bundle; *as*, accessory sepal trace.

The vascular bundles of the calyx are extensions of three whorls of traces. In flowers which have five sepals the median portion of each contains branches of a main sepal trace, while bundles in the lateral portions of each are connected with branches of adjacent petal traces and with accessory sepal traces. The five to eight bundles present at the base of each sepal are branched and anastomosed sparsely along their courses through the body of the sepal. Cells of the mesophyll are compact near the apexes of the sepals but near their bases are separated by large intercellular spaces. A few oil glands occur on the abaxial sides of the sepals.

Corolla

The petals originate as a whorl of crescent-shaped primordia. They alternate with the sepals and develop individually (figs. 15, 18). Their epidermal cells early become papillose, especially near the apices and edges. These papillae result in overlapping and interlocking of the petals, so that the corolla forms a closed chamber. Opening of the corolla is accompanied by shrinkage and plasmolysis of these papillose cells. In petals of a mature flower the cells of the mesophyll are isodiametric, and small intercellular spaces are abundant.

The five to eight bundles present in the base of a petal are branched repeatedly and anastomosed near the margins. These bundles are branches of two whorls of traces in the receptacle. Bundles of the median portion of each petal are connected directly with a median branch of a petal trace, while the lateral bundles of a petal are connected with lateral branches of adjacent sepal traces. While this is doubtless the fundamental pattern of vascular supply of the petals, and is in agreement with the findings of others (38, 56, 61), great variation exists. In corollas with three or four petals several branches from two adjacent petal traces may be present in one petal. Even in corollas with five petals, which is assumed to be the ancestral state, the bundles of the lateral portion of a petal may be connected with a complex of branches of sepal and petal traces, so that the true attachment is not discernible.

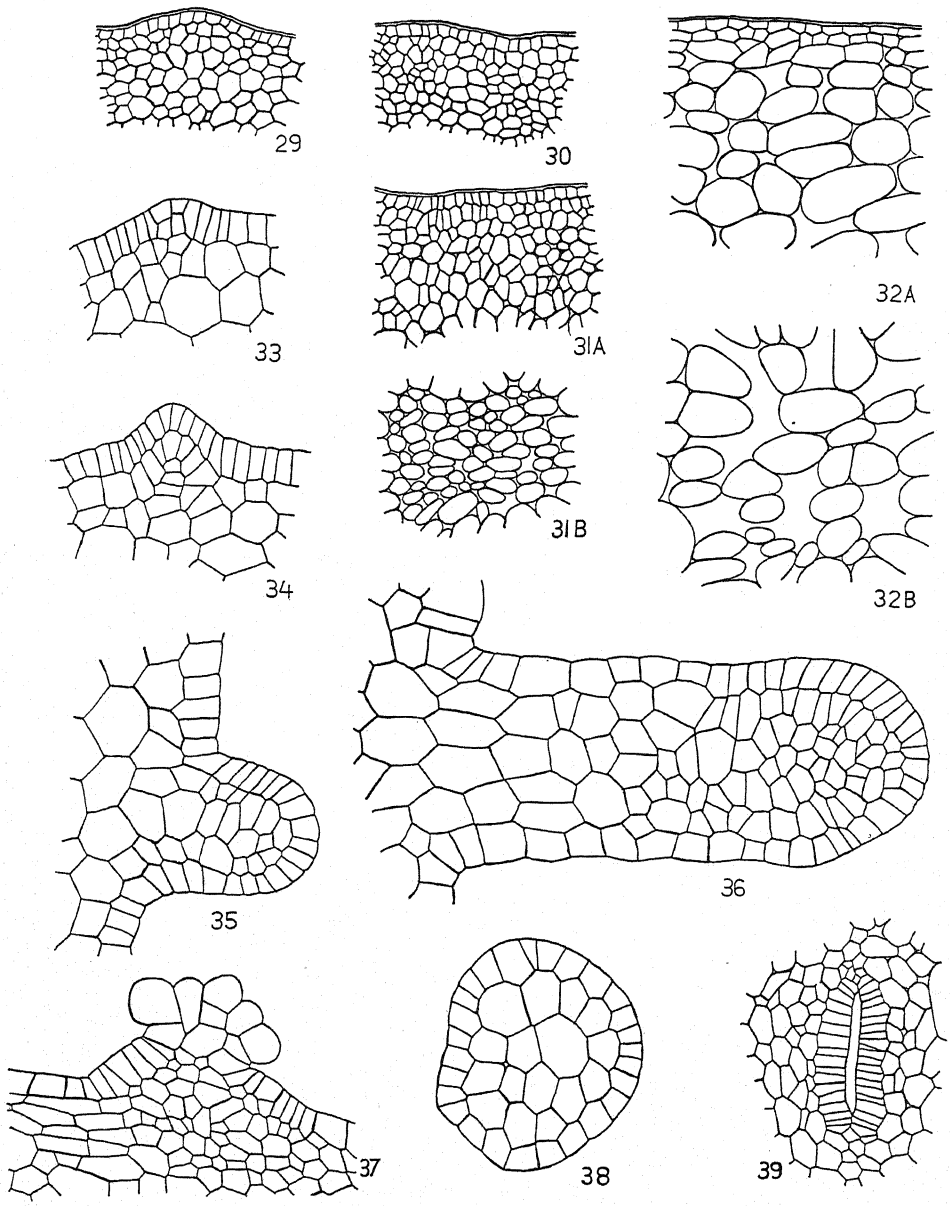
Stamens

The stamens originate after the petals have grown sufficiently to arch over the apex of the axis. Stamen primordia are dome-shaped and in one whorl. Although numerous young stages were observed, there was no evidence that the primordia of episepalous stamens originate before the other ones, in the manner reported for *C. aurantium* (37, 38). While some of the twenty-five or more stamens are separate almost to their bases, others are joined laterally in groups of three to seven, and only the upper 3–4 mm. of the filaments is separated (15, 28, 48, 49). Some filaments are noticeably longer than others, but they are not correlated with definite positions.

Each stamen has a single vascular bundle which extends into the anther. In the Eureka lemon the stamen traces all diverge from the vascular axis in a zone of 12 μ, and this can hardly be conceived to be two nodes. While this observation is in accord with other data (37, 56, 61), Penzig (38) considered the stamens of three other species of *Citrus* to arise in two whorls discernible by two whorls of vascular traces.

Pistil

Early ontogeny.—The carpels originate as a whorl of about nine crescent-shaped primordia located laterally near the apex of the axis. Cell multiplication



FIGS. 29–39.—Figs. 29–32*B*, section of ovary wall and pericarp in stages from prebloom to mature fruits, with diameters of 0.65, 4, 13, and 45 mm. Figs. 31*B* and 32*B* taken from inner pericarp adjacent to dorsal carpel bundles. Figs. 33, 34, primordia of juice sacs from radial longitudinal section of ovary of unopened flowers. Fig. 35, young juice sac as seen in longisection of ovary at time petals abscise. Fig. 36, median section lengthwise of juice sac from young fruit 10 mm. in diameter. Fig. 37, median longisection of one of carpellary emergences which failed to develop into juice sac; from fruit 24 mm. in diameter. Fig. 38, cross-section of juice sac at same age as fig. 36. Fig. 39, cross-section through stylar canal of mature flower. Cf. older stage, fig. 43.

occurs between bases of the primordia simultaneously with that in the primordia and the axis in such a way that the carpels develop with their margins undiverged from the margins of adjacent carpels or the axis. In early stages, therefore, the carpels are chambers open at the top (figs. 19, 20). At first the abaxial portions of the carpels grow faster than the adaxial portions and the axis, resulting in the formation of a depression in the apex of the developing pistil (fig. 22). The terminal portions of carpels develop into the stigma, while the style is developed from the apex of the receptacle, together with the upper portions of the adjacent carpel tissue. During elongation of the carpels the tops of the chambers become constricted into stylar canals (figs. 22–25). General meristematic activity of cells of the lower portions of the carpels and adjacent axis enlarges the locules. Early in their ontogeny ovule primordia develop on both margins of the carpels, and as the locule is increased in length new primordia originate below and above those formed earlier. At the time the flower opens the pistil consists of a receptacular core undiverged from the whorl of carpels. The placentation is axile, and a stylar canal extends from each locule to the stigma.

VASCULAR BUNDLES.—The dorsal carpel bundles differentiate early, and they are represented by one strong bundle for each carpel. They occur in the ovary wall at positions opposite the locules and in the outer part of the style opposite the stylar canals. The dorsal carpel bundles terminate just below the stigma (fig. 28). The marginal (ventral) carpel bundles differentiate at about the same time. They diverge from the receptacular bundles at the level of the center of the ovary and may equal the number of locules (fig. 9). Marginal carpel bundles are located in the axis of the ovary at positions opposite the septa, then are curved outward near the tops of the locules, where each anastomoses with a lateral carpel bundle (figs. 10, 11) and forms one concentric bundle which extends to the stigma (figs. 11, 12, 28). Lateral carpel bundles differentiate after the dorsal bundles. They are opposite the septa, curve inward, and are anastomosed with marginal bundles near the tops of the locules. In many instances the lateral bundles are in pairs along parts of their courses, but elsewhere the two bundles of the adjacent carpels are undiverged. The dorsal and lateral carpel bundles are much branched. These lesser vascular bundles extend outward at various angles from a complex network. Free ends of the latter bundles occur in the outer part of the ovary wall, and none of them extends into the style. At the time the flower opens the various bundles of the ovary are not well differentiated, and their histology is discussed in connection with the fruit.

OVARY.—When the flower opens the ovary is about 3 mm. in diameter and 5 mm. in length. The ovary wall consists of the median portions of the lower parts of the carpels and is about thirty cells thick. The outer cells of the ovary contain plastids which develop chlorophyll soon after the flower opens. Parenchyma of the middle portion of the ovary wall contains intercellular spaces but elsewhere is compact.

Oil glands are numerous in the outer part of the ovary wall. They are first detected as groups of cells whose cytoplasm is very dense and whose walls have begun to separate. Further development of an oil gland involves disintegration of a group of cells and formation of a cavity containing oil and other products. Large glands may extend halfway across the

ovary wall, as in other species of *Citrus* (8, 20, 58, 59). First initiation of glands takes place early in the development of the carpels and not after the flower is open, as reported for a Russian variety of Mandarin (20). Oil glands also occur in all other parts of the flower except the disk and the stamens, as others have noted (60). They are not associated with the adaxial surface of carpels, although BONAVIA (9) considered the juice sacs to be such.

The septa are composed of compact parenchyma and epidermis of adjacent carpels.

The lower part of the axis of the ovary consists of compact parenchyma and the receptacular bundles and placental traces. In the upper part the parenchyma is less compact, and in addition to the marginal carpel bundles stylar canals are present, the epidermal cells of which have dense protoplasts (fig. 39).

The anatropous ovules are turned in various directions. Some of their bundles diverge from marginal carpel ones, while the lower bundles usually diverge from the receptacular ones below the points where marginal carpel bundles diverge. The stylar canals are continuous with the locules above the bases of the uppermost ovules, and there are hairlike outgrowths of the epidermal cells at this point (fig. 41), each consisting of one slender cell or a filament of a few cells.

STYLE.—The style is composed of small unelongated parenchyma among which occur the two alternating whorls of vascular bundles, a few peripheral oil glands, and the stylar canals (figs. 11, 12). All pollen tubes observed were located in stylar canals.

STIGMA.—The stigma is globose and usually has an irregular central cleft. Cells at the surface are deeply staining and elongated at right angles. Near the edge of the stigma these cells comprise only one layer, but over the apex the stigmatic surface is composed of parallel filaments of two or three cells. In some stigmas oil glands occur just underneath the surface, near the ends of vascular bundles.

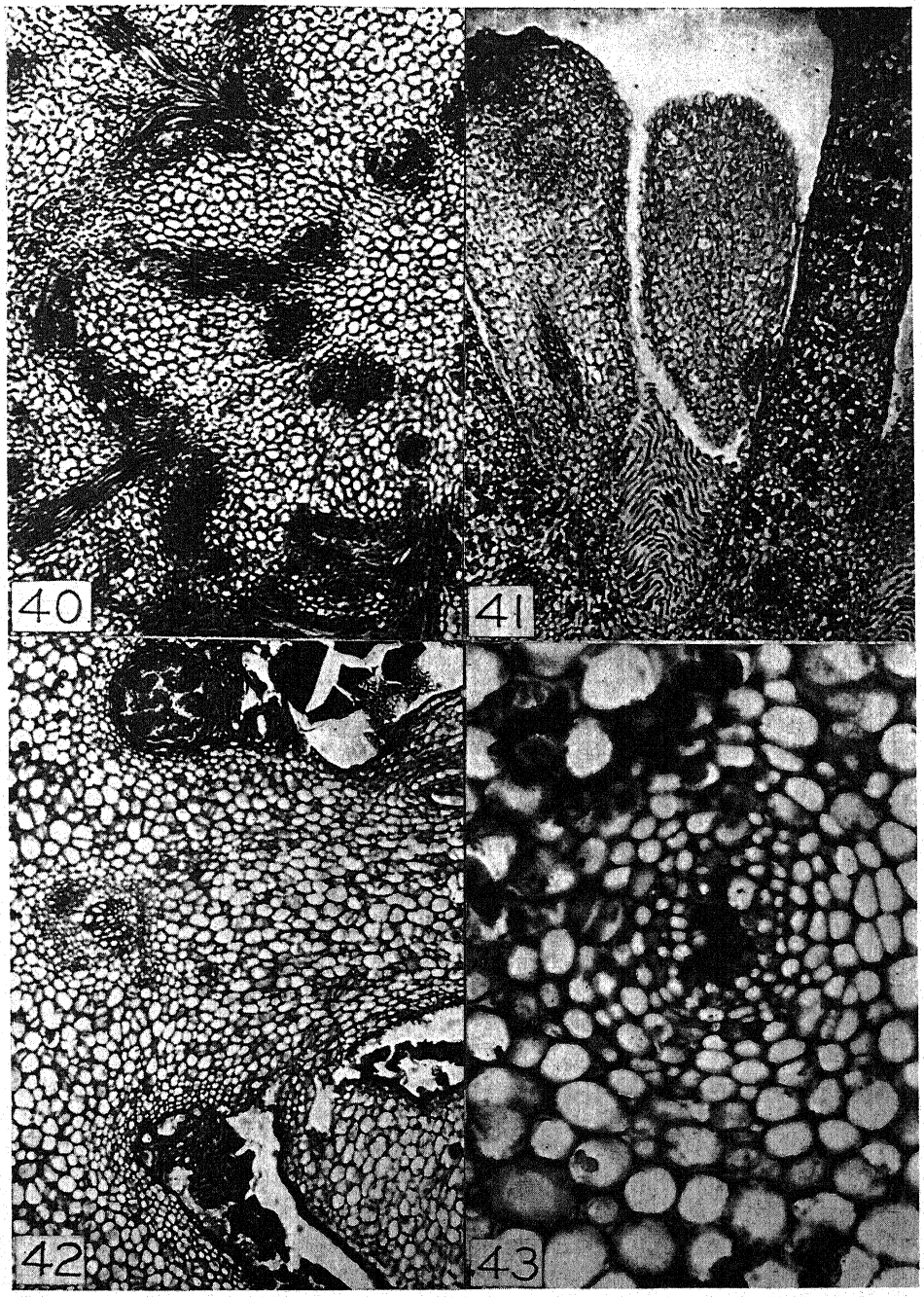
GYNOECIAL DISK

About the time the carpels begin to constrict at their tops, a ring of tissue above the bases of the stamens and below the carpels becomes meristematic and develops a massive, somewhat cup-shaped structure, the gynoecial disk, which completely encircles the base of the ovary (fig. 28). It is compact, stains densely, and its cells are exceedingly small and thin walled. Guard cells are present in its epidermis. Numerous small vascular strands are present (figs. 6, 7) and, contrary to reports (56) for other species of *Citrus*, usually are not attached directly to the vascular cylinder or to stamen traces, but to lateral carpel traces. This nectary does not develop further.

FRUIT

After the petals and sepals abscise, meristematic activity continues in various parts of the ovary. The top of the style disintegrates, and the fruit then develops from the ovary and base of the style (42).

RIND.—Cell multiplication in the ovary wall proceeds rather uniformly until the fruit is 10 mm. in diameter and the pericarp 3 mm. thick. In such young fruits the cells of the pericarp are thin walled, and intercellular spaces are minute and limited to the middle portion, where cells on the whole are twice the size of those of the outer and inner few layers. In subsequent stages the pericarp differentiates into three zones: an outer one of compact cells containing the



FIGS. 40–43.—Cross-sections. Fig. 40, of receptacle of flower bud showing one petal trace emerging from axis at position alternate with sepal traces which are branched into three strands each. Fig. 41, of ovary of open flower showing placental hairs at junction of stylar canal. Fig. 42, two undiverged inverted marginal carpel bundles. Mucilage occurs among placental hairs. Fig. 43, of stylar canal from apex of young fruit showing mucilaginous content of space. Cf. younger stage, fig. 39.

oil glands, a middle zone of spongy tissue, and an inner pericarp which is only a few cells thick and is attached to the juice sacs. Aside from the vascular bundles and epidermis, the whole pericarp is parenchymatous. The rind consists of the outer two zones of the pericarp, and although it does not separate easily from the pulp of the fruit, as does that of certain other species of *Citrus*, it is histologically distinct.

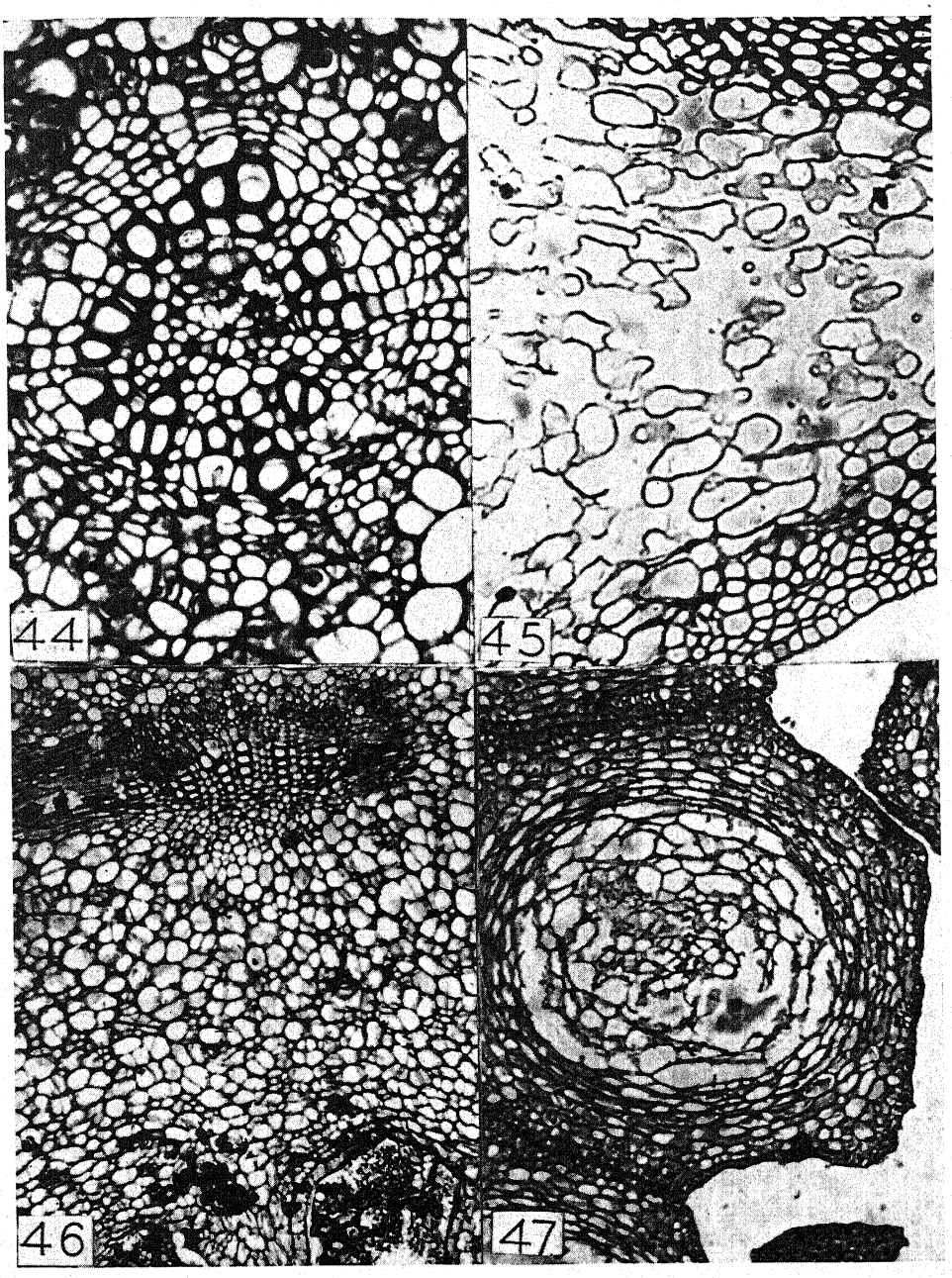
Stomata are present but are not abundant in the epidermis. Cells just underneath the epidermis are small and compact (figs. 29–31), but there is progressive increase in their size and of intercellular spaces farther inward (fig. 31*B*). These cells are isodiametric until mature, when they are slightly elongated parallel to the epidermis (fig. 32*A*). Oil glands are abundant underneath the epidermis, but they usually are separated from one another by eight or more parenchymatous cells, and many vascular bundles end near these glands. While some of the glands are formed during the early stages of development of the carpels, many new ones continue to develop, until the fruit is 15–20 mm. in diameter.

The spongy layer is notably developed (fig. 32*B*) when the fruit is 25 mm. in diameter and occupies about one-third of the width of the pericarp. Parenchymatous cells of this area are very thin walled and before the fruit is mature assume irregular shapes resembling leaf mesophyll. Intercellular spaces in mature fruits appear to equal or exceed the volumes of the cells. The demarcation between this zone and the outer one is gradual, but change to the small compact cells of the inner pericarp is abrupt.

All the vascular bundles of the ovary wall occur in the rind. During development there is increase in number as well as in size and differentiation of all the bundles. The dorsal and lateral carpel bundles change most. In young fruits these bundles are collateral and a cambium is present (fig. 46). But after the fruit is about 12 mm. in diameter the parenchymatous cells adjacent to the xylem portions of the vascular bundles become progressively meristematic toward the protoxylem of each bundle, and from their derivatives additional phloem, xylem, and cambial elements are differentiated (fig. 44).

INNER PERICARP AND JUICE SACS.—The inner pericarp is developed from the outer adaxial epidermis of the carpels, together with a narrow band of subepidermal cells. It is merged with the outer parts of the septa. The epidermal cells are similar to those of the outer pericarp, except that cutin is very thin and stomata are absent. The parenchyma adjoining the epidermis is compact and there are no oil glands. This zone contains no vascular bundles, although dorsal and lateral carpel bundles occur just outside it.

Development of the inner portion of the pericarp includes formation of the club-shaped emergences, the juice sacs, which extend into the locule. Primordia of these structures appear just before the flower opens, as reported for *C. aurantium* (41, 5). Their initiation after flowering has also been reported (18, 31). At first a primordium consists of a group of about five epidermal cells which have elongated radially and formed a small mound protruding into the locule. Primordia were observed only along the outer boundary of the locules and not on the septa. PENZIG (38) found the same condition in orange, but in *C. hystrix* he observed juice sacs attached to outer parts of the septa. In early stages of growth anticlinal divisions of epidermal cells of the primordium and division of



FIGS. 44–47.—Cross-sections. Fig. 44, of marginal carpel bundle from fruit 13 mm. in diameter. Cambium has become a cylinder and secondary xylem and phloem are present on all sides of primary xylem. Fig. 45, of inner portion of septum of mature fruit. Fig. 46, of portion of inner pericarp of 13 mm. fruit, showing position of dorsal carpel bundle and bases of juice sacs. Fig. 47, of juice sacs from fruit 24 mm. in diameter.

subepidermal cells take place. Early periclinal divisions of the epidermis were observed, but the emergence does not originate wholly from the epidermis (figs. 33, 34). During elongation of juice sacs there are repeated periclinal divisions of the epidermal cells at the tip, so that most of its cells are of epidermal origin. Young juice sacs are cylindrical and about five cells in diameter (figs. 35–38). They grow across the locules and project among the developing ovules when the fruits are 10 mm. in diameter. Each developing sac, at some point along its length, increases in diameter two or more times by cell multiplication in that area (fig. 47). This produces the familiar club shape of the emergence. There is little cell multiplication after the fruit is 20 mm. in diameter, however, and later enlargement of juice sacs results from increase in cell size. In mature fruits parenchymatous cells at the center of the juice sacs have very thin walls with minute intercellular spaces. Protoplasts have large vacuoles, and, by the use of Sudan 4, oil substances were detected in the protoplasts, as reported by DAVIS (14), as well as in the spaces formed when some cells break down. The epidermal cells which bound the mature juice sac are small, and many of them have thickened cell walls when the fruit matures.

Not all primordia develop into juice sacs, but some enlarge only slightly and their terminal cells become enlarged and globose, and cell divisions cease (fig. 37). TSCHIRCH (58) thought these papillose cells were the source of the mucilage so abundant in the locule of the young fruit of *C. vulgaris*, but Eureka lemon shows no evidence that mucilage is secreted from abortive emergences any more than from the developing juice sacs. Mucilage was not found in the locules of older fruits.

SEPTA.—Cells of the septa are markedly uniform, and cell multiplication continues until the fruit is some 20 mm. or more in diameter, when intercellular spaces become conspicuous in the median portions of the septa. This band of spongy parenchyma is merged on either side with the compact subepidermal cells and the epidermis of the carpels. In mature fruits, cells of the spongy area are stretched radially into long ellipsoidal shapes and are distinctly individual (fig. 45).

CORE.—The core develops from the axial portion of the ovary. Cell divisions increase the number to about 150 cells across the axis at the level of the placenta. In fruits that have attained 15 mm. in diameter small intercellular spaces begin to appear among the parenchymatous cells, and in subsequent development the central area of the core becomes spongy, especially above the level of the divergence of marginal bundles, where the spaces between vascular bundles is greatest. Similarly there is great development of intercellular spaces between the vascular bundles and the locules. This spongy area is continuous with that of the septa (fig. 45).

All the conducting hairs at the juncture of the stylar canals and locules become coated with mucilage and begin to decompose by the time the fruit is about 20 mm. in diameter (fig. 42). The core includes the lower parts of the stylar canals. Cells lining the canal itself usually do not break down with maturation of the fruit, but their cells become less compact. Their protoplasts become much less dense and resemble those of adjacent parenchyma (fig. 43). The canal itself contains mucilaginous material.

Vascular bundles of the lower part of the core increase in size. Fascicular cambium produces several rows of sec-

ondary tissues, and interfascicular cambium is produced between some of the bundles. Lignified tissues include a few annular elements, but for the most part they are composed of spiral tracheids 0.1–0.5 mm. in length. The thin-walled elements are elongated cells that stain densely, but no sieve plates were found. Above the ends of the axial bundles the marginal carpel bundles occur in the outer part of the core. In their early development they are endarch collateral, but during maturation they are changed by the same kind of cambial activity described previously in the dorsal and lateral bundles. The development of this condition occurs first toward the top of the locules. The ovule traces, which at blossoming time are minute, develop into conspicuous concentric bundles when the fruit is mature.

Discussion

Flower

While the flower of *Citrus* conforms with none of Shaffner's (47) floral types, it is not a complex one; yet in the literature several features have led to confusion in the application of morphological criteria.

The fact that the perianth is supplied by three series of vascular bundles rather than by two from the vascular axis raises a question in regard to the accessory sepal traces. If they are considered vestiges of other flowers of the inflorescence, in agreement with the ideas of Arber (2), then the concept of the flower as a pseudanthium is involved. Although this interpretation is conceivable, it seems unnecessary in the case of the lemon. Accessory sepal traces might be considered merely secondary bundles arising in connection with the enlarged receptacle. The fact that their provascular elements are present as early in ontogeny as those of the receptacular bundles would not favor this idea. A third possibility is that these bundles represent lateral or marginal sepal traces. This concept is supported by the facts that they occupy normal positions for such bundles and that a few of them were found attached to bundles that diverged as main sepal traces. It may be unusual for lateral traces to diverge from the vascular cylinder below the main dorsal ones, but that condition has been described by Penzig (38) in leaf traces of certain oranges and seems also to be true of the lateral abaxial bundles of the carpels of Eureka lemon.

It has been assumed by Tillson and Bamford (56) that the stamens in *Citrus* belong to more than one whorl. Payer (37) and Penzig (38) thought there were two whorls in *C. aurantium*. But in Eureka lemon it has not been possible to observe either the three ages of stamen primordia or two whorls of stamen traces which the latter investigators used as evidence. The coherence of stamens in indefinite groups does not shed light on the issue, and the criterion of alternation cannot be used in this regard in lemon because of the number of carpels. In complete agreement with van Tieghem (61), observations on the anatomy of the lemon indicate there exists one whorl of stamens, although in a flower with thirty stamens they conceivably may represent two whorls of five single stamens and two whorls of five forked stamens, all "compressed" into one level. To apply Wilson's (63) idea of primitiveness of forked stamens in the interpretation of the stamens of Eureka lemon would add to the confusion.

Opinions concerning the cup-shaped nectaries of *Citrus* have followed two widely different lines. Partly because they found its vascular bundles attached to stamen traces, Tillson and Bamford

(56) considered the so-called gynoecial disk to represent a vestigial stamen whorl. This view seems not to be substantiated by the facts that in Eureka lemon many, if not most, of the bundles present in this structure are connected with those of the main receptacle or with the lowermost carpel traces, and also that it originates after the carpels have attained considerable size. If the gynoecial disk is a vestigial structure, there is no more evidence for considering it as derived from stamens than from another whorl of carpels. More in accord with anatomical facts is the view that the disk arises merely as a proliferation of the axis, owing to continued meristematic activity accompanying growth of the gynoecium. This might account for the indefinite attachment of the vascular bundles supplying this structure and for its origin out of acropetal order. Views similar to this were held by BAILLON (5), PAYER (37), PENZIG (38), and others.

In the foregoing description the tacit assumptions have been that the flower represents the terminal portion of one shoot and that the carpels are foliar in nature, in the manner expressed by KOZO-POLJANSKI (30), PARKIN (36), and NEWMAN (34). Nothing has been observed in the developmental anatomy of lemon to make it desirable to apply the concept of sterile and fertile carpels of SAUNDERS (45) nor to resort to the so-called "new morphology" of THOMAS (52) or THOMPSON (53, 54, 55), who deny the existence of the carpel as such. Other contradictory interpretations regarding the angiosperm carpel are those of TROLL (57) and GREGOIRE (24), and it is not pertinent here to discuss them, for that has been done by others (4, 6, 21, 33, 64, 30, 3). GREGOIRE, however, has erred in one anatomical point, for in the fruit of Eureka lemon differentiation of the carpel is not different from that of a foliage leaf, either in regard to the vascular bundles or to other tissues.

How much of the gynoecium is composed of axis tissue is of interest in species of *Citrus* because various workers, notably SAUNDERS (46) and OSAWA (35), have alluded to the prolongation of the axis in the Navel orange, and others (7, 17) have referred to this condition in other families. On evidence of ontogeny and the positions of the bundles in the style, the axis might be conceived to extend well up the style and to occupy there most of the space surrounded by the stylar canals. But there is no way to distinguish accurately how much of this tissue is carpel margins, and there is little residual vascular tissue in the Eureka lemon, and none of it extends into the style.

The epidermis of the stylar canals leads from the stigma to the placental surface of the locules. It is therefore strange, as ARBER (3) and TILLSON and BAMFORD (56) point out, that JOSHI (27) considered these structures in angiosperms generally to be vestigial vascular bundles. Because of the stylar canals, each carpel has the superficial form of a cupule whose terminal portion is constricted, as suggested by THOMAS (52) for carpels of certain simpler pistils; but this fact does not indicate homology with such structures present in the Caytoniales.

Placental areas offer no obstacle to a foliar interpretation of the carpels. The origin of younger ovules, both above and below the older ones, is explained by the general meristematic activity during development of the ovary as a whole. The continuity of the vascular bundles of the lowest ovules with the receptacular bundles is directly related to adnation of carpel margins to the receptacular axis

and does not require their interpretation as axial ovules, as BUGNON (10) did for those in *Begonia*. The hairs at the juncture of the stylar canal are similar in nature to the papillose epidermal areas of the petals and the stigma.

The coherence of vascular bundles of adjacent carpels is pronounced, both as regards marginal and lateral (septal) carpel bundles, but the dorsal carpel bundles diverge from the vascular cylinder above the lateral carpel bundles. This might be expected in *Citrus*, since the traces of a foliage leaf—according to PENZIG (38)—diverge in a similar manner; that is, lateral leaf traces diverge from the vascular cylinder below the level where the main dorsal leaf traces are separate from the vascular cylinder.

The presence of concentric bundles in the style need not be confusing when it is recalled that each is an extension of two bundles; and one of these, the marginal carpel bundle, has its xylem and phloem in inverted positions because of the position of the margins of the carpels.

FRUIT

To consider the rind of lemon a torus, as DE CANDOLLE (15) did for certain species of *Citrus*, contradicts all concepts of the angiospermous carpel except those of SAUNDERS (45), because most of the carpel bundles occur in the rind. The same may be said of BONAVIA's (9) concept of the rind as a whorl of sterile carpels. It is improbable that either worker conceived carpels to be polymorphic, but it seems much more likely that their ideas were based upon the ease with which the rind separates from the pulp, and that they ignored the vascular anatomy of the fruit.

When the structure of the rind is viewed in comparison with that of a foliage leaf, it becomes evident that the line where rind can be separated from the inner pericarp coincides with the spongy mesophyll tissue present in many foliage leaves. The fact that this zone of intercellular space development in the carpels of lemon is near the upper rather than the lower epidermis can be explained on ecological bases. The separation of the sectors of the pulp from one another and from the core likewise is made possible by the copious development of intercellular spaces adjacent to the inner epidermis of the carpels. The small amount of cell disintegration observed along these lines of cleavage seemed coincidental with cell separation rather than with other causes involving secretion of specific enzymes.

It is perhaps unwise to homologize juice sacs with epidermal hairs, because this may obscure the fact that juice sacs are derived from more than the epidermis of the carpel, a fact well established in at least three species of *Citrus* by POULSEN (41), PENZIG (38), BIERMAN (8), and by the present observations. It is also improbable that the juice sacs are derived from oil glands (9). Presence of oil in cells of the juice sacs is similar to other cells of the pericarp, and the occurrence of spaces in older juice sacs is probably due to tension resulting from osmotic forces. There are no structures of Eureka lemon with which the juice sacs may be homologous.

Summary

1. In Eureka lemon the succession of the whorls of the flower parts is acropetal. The nectary or gynoecial disk originates after the carpels.

2. The courses of the vascular bundles and the general histological features of the floral parts are described.

3. The gynoecium is considered a whorl of carpels around the apex of the

receptacle which extends past the level of the placenta.

4. Development of the fruit involves a period of cell multiplication throughout the ovary, followed by one of cell differentiation. Differentiation in the pericarp produces three zones: (*a*) an outer exocarp which is compact and contains oil glands, the flavido; (*b*) the mesocarp which is spongy and becomes most highly developed close to the inner pericarp, the albido; (*c*) the inner pericarp which is compact and from which the juice sacs diverge.

5. Primordia of juice sacs appear just before opening of the flower and occur on the adaxial surface of each carpel. After the juice sacs grow across the locules, a portion of each enlarges greatly in diameter. Cells in the center of these areas contain oil and are extremely delicate. Spaces containing gelatinized material in the juice sacs are considered incidental to high turgor and not to action comparable to gland formation.

6. Separation of the sectors of the pulp from one another and from the rind and core is made possible by cell separation and the formation of a spongy area in the tissue adjacent to the adaxial surfaces of the carpels.

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LITERATURE CITED

1. ABBOTT, C. E., Blossom-bud differentiation in *Citrus* trees. Amer. Jour. Bot. **22**:476–485. 1935.
2. ARBER, AGNES, Studies in flower structure. VI. On the residual vascular tissue in the apices of reproductive shoots, with special reference to *Lilaea* and *Amherstia*. Ann. Bot. **4**:617–627. 1940.
3. ———, The interpretation of the flower. A study of some aspects of morphological thought. Biol. Rev. Cambridge Phil. Soc. **12**:157–184. 1937.
4. ———, Floral anatomy and its morphological interpretation. New Phytol. **32**:231–242. 1933.
5. BAILLON, H. E., De la famille des Aurantiacées. Thèse pour le doctorat en médecine. Paris. 1885.
6. BANCROFT, H., A review of researches concerning floral morphology. Bot. Rev. **1**:77–99. 1935.
7. BERGMAN, H. F., Intracarpellary fruits and other central proliferations of the floral axis in *Hibiscus*. Amer. Jour. Bot. **19**:600–603. 1932.
8. BIERMAN, M., Beitrage zur Kenntnis der Entwickelungsgeschichte der Früchte von *Citrus vulgaris* Risso, und anderer Citrusarten. Inaugural dissertation. Univ. Bern. 1896.
9. BONAVIA, E., The cultivated oranges and lemons of India and Ceylon, with researches on their origin and derivation of their names. London. 1888.
10. BUGNON, P., Les bases antomique de la théorie de la concrescence congénetale. Bull. Soc. Bot. France **75**:25–33. 1928.
11. CARUEL, F., Observations sur la nature et l'origine de la pulpe qui entoure les graines dans certains fruits. Ann. Sci. Nat. Bot. IV. **12**:72–77. 1859.
12. CLOS, M. D., Observations sur le pistil ou le fruit des genres *Papaver* et *Citrus*. Ann. Sci. Nat. Bot. V. **3**:312–320. 1865.
13. CRUEGER, K., Ueber Befrüchtung bei Orangers. Bot. Zeitschr. **4**:57–63; **5**:73–80. 1851.
14. DAVIS, W. B., Deposits of oil in the juice sacs of citrus fruits. Amer. Jour. Bot. **19**:101–105. 1932.
15. DE CANDOLLE, AUGUSTIN, Organographie végétale. Vol. II. Paris. 1827.
16. DE CANDOLLE, ALPHONSE, Origin of cultivated plants. Pp. 178–188. New York. 1885.
17. DICKSON, JEAN, Studies in floral anatomy. An interpretation of the gynaecium in the Primulaceae. Amer. Jour. Bot. **23**:385–393. 1936.
18. DUCHARTRE, P. É. S., Éléments de botanique. p. 647. Paris. 1867.
19. DUFRENOY, JEAN, A cytological study of water-soluble and fat-soluble constituents of *Citrus*. Jour. Agr. Res. **38**:411–429. 1929.
20. DZHAPARIDZE, L. I., and KEZELI, T. A., Razvitie efirnomaslichnykh Vmestilishch v Kozhure mandarina. (Development of the oil glands in rind of *Citrus unschin.*) With English summary. Botanicheskii Zhurnal USSR **20**:131–136. 1935. (Biol. Abstr. **11**:9585. 1937.)
21. EAMES, A. J., The vascular anatomy of the flower with refutation of the theory of carpel

polymorphism. Amer. Jour. Bot. 18:147–188. 1931.
22. FROST, H. B., Polyembryony, heterozygosis, and chimeras in *Citrus*. Hilgardia 1:365–402. 1926.
23. ———, Nucellar embryony and juvenile characters in clonal varieties of *Citrus*. Jour. Hered. 29:423–432. 1938.
24. GREGOIRE, V., La morphogénèse et l'autonomie morphologique de l'appareil floral. I. Le carpelle. La Cellule 47:287–452. 1938.
25. GREW, NEHEMIAH, The anatomie of plants with an idea of a philosophical history of plants. pp. 180–181. London. 1682.
26. IKEDA, T., On parthenocarphy in *Citrus*. Japanese Jour. Sci. Agr. Soc. Nos. 60, 63. 1904; no. 70. 1905.
27. JOSHI, A. C., The morphology of the stylar canal in angiosperms. Ann. Bot. 48:967–974. 1934.
28. KERNER, A., and OLIVER, F. W., The natural history of plants. Vol. I. London. 1902.
29. KEZELI, T. A., On the anatomy of the rind of Mandarines from Georgia. With English summary. Trudy Tbiliss. Botanichesk. Inst. 4:155–161. 1938. (Biol. Abstr. 14:3327. 1940.)
30. KOZO-POLJANSKI, B., On some "third" conceptions in floral morphology. New Phytol. 35: 479–492. 1936.
31. LICOPOLI, G., Sul frutto del melarancio edel Limone. Nuov. biorn. Bot. Italano 9:110. 1877.
32. MASTERS, M. T., Vegetable teratology. pp.75, 303. London. 1869.
33. MOORE, J. A., Floral anatomy and phylogeny of Rutaceae. New Phytol. 35:318–322. 1936.
34. NEWMAN, IV., Ontogeny of the angiospermic carpel. Nature 137:70–71. 1936.
35. OSAWA, I., Cytological and experimental studies in *Citrus*. Jour. Coll. Agr. Imp. Univ. Tokyo 4:83–116. 1912.
36. PARKIN, J., The classical carpel and recent attacks. Rept. Bot. Exchange Club of Brit. Isles 10:598–606. 1933.
37. PAYER, J. B., Traité d'organagenie comparée de la fleur. pp. 112–117. Paris. 1857.
38. PENZIG, O., Studi botanici suggli agrumi e sulle piante affini. Memoria Premiata Minis. Agric. Con Atlante (Dagli Ann. Agric.). Roma. 1887.
39. ———, Pflanzen teratology systamatisch geordnet. Vol. II. Berlin. 1922.
40. POORE, H. D., Analyses and composition of California lemons and orange oils. U.S. Gov. Printing. 1932.
41. POULSEN, V. A., Pulpaens undvikling hos *Citrus*. Botaniska Notiser. 4:97–103. 1877.
42. REED, H. S., Certain relationships between the flowers and fruits of the lemon. Jour. Agr. Res. 17:153–165. 1919.
43. RISSO, A., and POITEAU, A., Histoire et culture des orangers. 2 vols. Paris. 1872.
44. ROSS, L. S., On the structure and development of lemons. BOT. GAZ. 15:262–267. 1890.
45. SAUNDERS, E. R., Floral morphology. A new outlook with special references to the interpretation of the gynaeceum. Vol. I. pp. 1–17, 84, 85. Cambridge. 1937.
46. ———, On carpel polymorphism. VI. Ann. Bot. 48:643–692. 1934.
47. SHAFFNER, J. N., The fundamental nature of the flower. Bull. Torr. Bot. Club 64:569–582. 1937.
48. STRASBURGER, E., Handbook of practical botany for the botanical laboratory and private student. pp. 427–429. New York. 1911.
49. ———, STRASBURGER'S textbook of botany. 5th English edition. p. 653. London. 1921.
50. SWINGLE, W. T., A new taxonomic arrangement of the orange sub-family Aurantioideae. Jour. Wash. Acad. Sci. 28:530–533. 1938.
51. TANAKA, T., The taxonomy and nomenclature of Rutaceae Aurantioideae. Blumea 2:101–110. 1936.
52. THOMAS, H. H., The nature and origin of the stigma. A contribution towards a new interpretation of the angiosperm flower. New Phytol. 33:173–198. 1934.
53. THOMPSON, J. M., On the place of ontogeny in floral enquiry. Publ. Hartley Bot. Lab., Univ. Liverpool 17:1–20. 1937.
54. ———, The acarpous nature of modern flowering. Proc. 6th Int. Bot. Cong. 2:122–124. 1935.
55. ———, The state of flowering known as angiospermy. Publ. Hartley Bot. Lab., Univ. Liverpool 12:1–47. 1934.
56. TILLSON, A. H., and BAMFORD, R., The floral anatomy of the Aurantioideae. Amer. Jour. Bot. 25:780–793. 1938.
57. TROLL, W., Organisation und Gestalt im Bereich der Blute. Gesantgebeit wiss. Bot. Monograph 1:1–413. 1928.
58. TSCHIRCH, A., and OESTERLE, O., Anatomischer Atlas der Pharmakognosie und Narhumgsmittelkund. pp. 302–306. Leipzig. 1900.
59. ———, Angewandte Pflanzenanatomie. Wien and Leipzig. 1889.
60. TURREL, F. M., On the cellular nature of the citrus juice sac. Calif. Citrograph 24:386. 1939.
61. VAN TIEGHEM, P., Recherches sur la structure du pistil et sur l'anatomie comparée de la fleur. Mem. Acad. Sci. Inst. Imp. France 21:1–261. 1868.
62. VOLKAMER, J. C., Nurmbergische Hesperides oder Gründliche Beschreibung der edlen citronat, citronen, und pomerantzen Früchte. Nuremberg. 1708.
63. WILSON, C. L., The morphology of the stamen. Amer. Jour. Bot. 24:686–699. 1937.
64. WILSON, C. L., and JUST, T., The morphology of the flower. Bot. Rev. 5:97–131. 1937.
65. ZUCCARINI, J. G., Ueber die Bildung des Fruchtfleisches bei der Gattung *Citrus*. Abhand, Math. Phys. Classe Konigl. Bayerischen Akad. Wissenschaften 4:33–35. 1845.

INFLUENCE OF SULPHUR DEFICIENCY ON METABOLISM OF BLACK MUSTARD

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 549

SCOTT V. EATON

(WITH ONE FIGURE)

Introduction

The writer has for some time been studying the effects of sulphur deficiency on the metabolism of plants. The project as originally outlined involved three plants. Since leguminous and cruciferous species are especially high in sulphur, the former on account of the high protein content and the latter owing to various sulphur compounds in addition to proteins, it was planned to study a representative of each family. And for comparison a plant from a third family was to be investigated. The three plants chosen after considerable preliminary work were the Manchu soybean, a representative of the Leguminosae; the black mustard, belonging to the Cruciferae; and the Mammoth Russian sunflower, a member of the Compositae.

The results of the effects of sulphur deficiency on two of the plants have already been published, the soybean (2) and the sunflower (3). Other phases of the subject which have been investigated are the effects of sulphur deficiency on the volatile sulphur content of the black mustard (5) and the relation of the sulphur content of the seed and seed weight to the effects of sulphur deficiency on the growth of the sunflower (4). The present paper reports the results for black mustard, *Brassica nigra* (L.) Koch.

Since this paper completes this entire project, the discussion section summarizes and compares the effects of sulphur deficiency on the growth and metabolism of plants, with special reference to the three plants already mentioned.

Methods

GROWING THE PLANTS.—In choosing a cruciferous plant for this study, kale, rape, and white mustard were grown with plus-sulphur and minus-sulphur nutrient solutions in connection with the work on the soybean (2). The weights of the tops and roots were obtained and the top-root ratios calculated, but no analyses were made. The effects of sulphur deficiency on black mustard and white mustard were compared in the summer of 1941 as part of the volatile sulphur experiment (5). Much greater reduction in growth was obtained in the case of the former species, and it was decided to use this plant.

The methods of growing the plants were essentially the same as previously described (3). The main modification made was that a more dilute nutrient solution was used. In growing the plants during the spring and summer for the volatile sulphur study (5), some of them developed certain chlorotic effects and malformations of the leaves which did not seem normal. So in the autumn of 1941 an experiment was tried which involved varying the concentration of the nutrient solution and also the frequency

of its application. The growth period was October 4 to November 14. The results are given in table 1.

Although, perhaps due to seasonal differences, the plants of none of the solutions—even the most concentrated—developed the abnormal characteristics just mentioned, the plants of the half-strength nutrient solution added daily are considerably larger than those of any other series. Since the plants were grown during the short days of autumn, however, it was thought best in the present work to use a somewhat stronger concentration, and a solution three-fourths of full strength was used. Table 2 gives its composition. Boric acid and manganese chloride at a concentration of 0.25 p.p.m. of each of the elements, boron and manganese, were kept constantly in the nutrient solution, which was added daily. A solution of ferric citrate at a concentration of 1.0 p.p.m. of iron was sprinkled on to the sand twice a week, and zinc chloride and cupric chloride, at concentrations of 0.05 and 0.02 p.p.m., respectively, of zinc and copper, were applied every 2 weeks.

TABLE 1

GREEN WEIGHT (GRAMS PER 25 PLANTS)

Solution	Tops	Roots	Entire plant
Full strength* added daily	55.5	10.3	65.8
Full strength added three times per week	58.0	11.3	69.5
One-half strength added daily	70.5	12.3	82.8
One-half strength added three times per week	57.0	8.3	65.5
One-fourth strength added daily	58.1	10.2	68.3
One-fourth strength added three times per week	42.5	6.8	49.3

* Full strength refers to a nutrient solution having an osmotic pressure of one atmosphere. The solution used was $1R_2S_4$ of the triangle (7). Because of the greater ionization, the diluted solutions would, of course, have an osmotic pressure greater in proportion to the full-strength solution than that indicated by the dilution.

TABLE 2

PARTIAL VOLUME MOLECULAR CONCENTRATION OF SALTS OF NUTRIENT SOLUTION

Solution	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	$MgCl_2$
Minus-S	0.0034	0.0068	.....	0.0034
Plus-S	0.0034	0.0068	0.0034	.....

The seeds were planted on April 8, 1942, and the plants harvested May 11.

CHEMICAL METHODS.—At the time of harvest some of the plants of each set had formed flower buds. None of these were included in the sampling. Two hundred and thirty minus-sulphur and 101 plus-sulphur plants were taken for analysis. Only the stems were analyzed, since previous work (2) indicated that sulphur deficiency affects the stems of plants more than other parts. The stems were cut up finely, and duplicate 100-gm. samples were weighed. The tissue was extracted with 70 per cent alcohol, as in the previous work (3). This seems to be an efficient solvent. In the case of the nitrogenous compounds, most of the nitrogen is found in the extract; there is little in the residue (table 4).

The methods used in the determination of the nitrogenous fractions were essentially as previously described (2, 3). The determinations were made promptly to avoid changes such as those described by WEBSTER (17) and STUART (15). PHILLIPS' (10, 11) methods were used in the determination of amides and nitrates, with a few modifications. Five per cent H_2SO_4 instead of 10 per cent as suggested by PHILLIPS was used in the hydrolysis of the amides, and the ammonia was aspirated off with 0.65 per cent HaOH, as given by DAVIDSON and SHIVE (1). Ten per cent H_2SO_4 seems a rather high concentration for amide hydrolysis. In the determination of nitrates, the boiling

down to a small volume after the transference of the extract to the Kjeldahl flask was not done because it was thought that ammonia might be liberated from some of the nitrogenous compounds, owing to the increased concentration of the alkali.

Volatile sulphur was determined by the method of PLATENIUS (12) and the other sulphur fractions as previously described (3).

Results

The effects of sulphur deficiency on the size of the plants developed rather quickly. In less than 2 weeks after planting, the minus-sulphur plants were shorter and the leaves smaller than the plus-sulphur. Chlorosis of the leaves developed late. It was not until about 1 week before harvest that this could be detected; then the young upper leaves showed the symptom to a marked degree. Up to this time the leaves of the minus-sulphur plants were even greener than the plus-sulphur. Anthocyanin development in leaves and stems, hairiness of these organs, the pim-

TABLE 3

GREEN WEIGHT (GRAMS PER 100 PLANTS), TOP-ROOT RATIO

SOLUTION	LEAVES	STEMS	ROOTS	ENTIRE PLANT	TOP-ROOT RATIO	PERCENTAGE MOISTURE OF STEMS	PERCENTAGE DRY WEIGHT OF STEMS
Minus-S.....	342.6	129.1	47.9	519.6	9.847	92.726	7.274
Plus-S.....	660.2	507.6	82.5	1250.3	14.152	95.051	4.949

TABLE 4

NITROGEN FRACTIONS (IN PERCENTAGES)

SOLUTION	DRY MATTER	GREEN MATTER	DRY MATTER	GREEN MATTER	DRY MATTER	GREEN MATTER	DRY MATTER	GREEN MATTER
	TOTAL N		TOTAL ORGANIC N		70% ALCOHOL-SOLUBLE N		70% ALCOHOL-INSOLUBLE N	
Minus-S.....	6.112	0.445	2.996	0.218	4.520	0.329	1.592	0.116
Plus-S.....	6.143	0.304	2.285	0.113	4.391	0.217	1.752	0.087
	AMMONIA N		AMINO N		AMIDE N		NITRATE N	
Minus-S.....	0.237	0.0172	0.452	0.034	0.219	0.016	3.116	0.227
Plus-S.....	0.077	0.004	0.114	0.006	0.106	0.005	3.858	0.191

The only modification made in the carbohydrate methods was in the permanganate titration of cuprous oxide. This was made at room temperature, with the indicator ortho-phenanthroline, rather than at a temperature of 80° C. as recommended by SULLIVAN (16). The indicator fades so rapidly at the high temperature that it is rather difficult to detect the end point. This does not occur at room temperature, and yet the permanganate reacts readily.

ply character of the younger leaves, and the hardness of the stems and the stiff texture of the leaves were also prominent effects of sulphur deficiency. Figure 1 shows the difference in the size of the plants at the time of harvest. The photograph does not do justice to the difference in color.

In a number of cases the tips and edges of the young leaves of the minus-sulphur plants bent downward. This was not noticed in the case of the previous plants (2, 3) and is not considered a characteristic symptom, although McMURTREY (8) states that sulphur deficiency frequently causes the tips of the leaves of the tobacco to crimp downward, and STOREY and LEACH (14) describe the uprolling of the leaves of the tea bush as characteristic of the yellows disease of this plant. Their work shows rather definitely that this disease is due to a deficiency of sulphur.

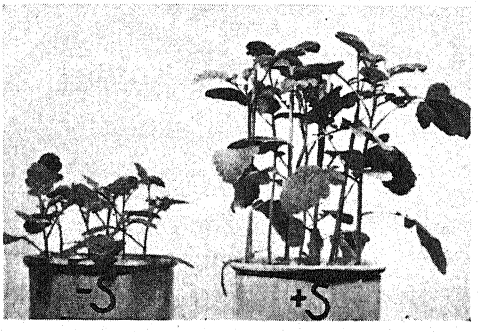


FIG. 1.—Black mustard plants, May 10, 1942; 32 days from planting of seed.

The indications were that sulphur deficiency caused earlier reproduction. It has been mentioned that at the time of harvest a number of the minus-sulphur and plus-sulphur plants had formed flower buds. A count showed that this had occurred in a higher percentage of the former than of the latter. The phenomenon had been noticed in preliminary work on both the black and white mustard. The matter should be studied more fully, using a greater number of plants. As stated, none of the plants that had formed flower buds were taken for analysis.

It is evident from table 3 that sulphur deficiency very much reduces growth of the mustard plant. The stems are affected much more than the leaves and the roots. The top-root ratio of the minus-sulphur plants is relatively low, showing that the weight of the tops is reduced more than that of the roots, but of the two organs of the tops, the ratio is due much more to the stems than to the leaves.

Tables 3–9 give the data for the chemical composition of the plant. Because of the big difference in the amount of dry matter in the two sets of plants, the percentages are figured on both dry-weight and green-weight bases. The data are also calculated on the absolute basis.

NITROGEN FRACTIONS

Calculated as percentage of green matter, the minus-sulphur stems contain more than four times as much ammonia, five times as much amino acids, and three times as much amides as the plus-sulphur stems (table 4). All the other nitrogen fractions are higher in the sulphur-deficient stems, although the nitrates accumulate only to a slight extent. When the data are calculated on the dry-weight basis the differences are less and are reversed in the case of total nitrogen, 70 per cent alcohol insoluble nitrogen, and nitrate nitrogen. The ammonia was determined by vacuum distillation and therefore includes any other volatile bases present.

Table 5 gives the data of the nitrogen fractions calculated on the absolute basis. The much larger plus-sulphur stems

contain more of all the nitrogen fractions except ammonia and amino acids. The fact that the much smaller minus-suphur stems contain more of these compounds emphasizes their accumulation as a result of sulphur deficiency.

Carbohydrate fractions

On the green-weight basis the minus-sulphur stems contain only about one-fifth as much reducing sugars as the plus-sulphur, but they contain almost four times as much sucrose, six times as much starch, and also somewhat more acid-hydrolyzable carbohydrates (table 6). On the dry-weight basis the difference in reducing sugars is intensified but reduced in the case of the other compounds.

When the data are calculated on the absolute basis (table 7), the large plus-sulphur stems contain more of all the carbohydrates except starch. The percentage of this carbohydrate is so much higher in the small minus-sulphur stems that they contain more of it than the much larger plus-sulphur stems.

Table 8 records the percentage data for volatile sulphur, for the sulphur left in the extract, and for the residue after removal of the volatile sulphur by steam distillation. The determinations do not represent duplicates. It was not possible to attend to more than two pieces of the volatile-sulphur apparatus at the same time, so one determination was made on each of the two sets of plants on each of two consecutive days. The plants were of the same crop but were necessarily different plants. One of the plus-sulphur determinations was lost.

On the dry-weight basis the minus-sulphur stems contain from less than one fifth to less than one-tenth as much volatile sulphur as the plus-sulphur stems. The differences on a fresh-weight basis are less. The two percentages for

TABLE 5

NITROGEN FRACTIONS (GRAMS PER 100 PLANTS)

Solution	Total N	Total organic N	70% alcohol-soluble N	70% alcohol insoluble N	Ammonia N	Amino N	Amide N	Nitrate N
Minus-S	0.574	0.281	0.425	0.149	0.022	0.042	0.021	0.293
Plus-S	1.543	0.574	1.103	0.440	0.019	0.029	0.027	0.969

TABLE 6

CARBOHYDRATE FRACTIONS (IN PERCENTAGES)

Solution	Dry matter	Green matter	Dry matter	Green matter	Dry matter	Green matter	Dry matter	Green matter
	Reducing sugars		Sucrose		Starch		Acid-hydrolyzable carbohydrates	
Minus-S	0.318	0.023	0.549	0.040	2.404	0.175	10.496	0.764
Plus-S	2.303	0.114	0.230	0.011	0.608	0.030	9.657	0.478

the sulphur-deficient stems do not agree very well, owing mainly to the very small amount of sulphur present. The determinations varied by only 2.5 mg. of barium sulphate or 0.34 mg. of sulphur. The sulphur in the extract, in the residue, and total sulphur are also much lower in the minus-sulphur than the plus-sulphur stems. No sulphur at all was found in the residue of the former and very little in the latter. On the absolute basis (table 9) the large plus-sulphur stems contain much more of each of the fractions. Considerable additional data on the effects of sulphur deficiency on the volatile sulphur content of the black mustard are given in a previous paper (5).

TABLE 7

CARBOHYDRATE FRACTIONS (GRAMS PER 100 PLANTS)

Solution	Reducing sugars	Sucrose	Starch	Acid-hydrolyzable carbohydrates
Minus-S....	0.030	0.052	0.226	0.986
Plus-S.....	0.579	0.058	0.153	2.426

TABLE 8

SULPHUR FRACTIONS (IN PERCENTAGES)

Solution	Dry matter	Green matter	Dry matter	Green matter	Dry matter	Green matter	Dry matter	Green matter
	Volatile S		S in extract		S in residue		Total S	
Minus-S.....	0.0045	0.0003	0.2171	0.0165	0.0000	0.0000	0.2216	0.0168
Minus-S.....	0.0088	0.0008	0.1229	0.0107	0.0000	0.0000	0.1317	0.0115
Plus-S.....	0.0480	0.0028	0.6450	0.0378	0.0355	0.0021	0.7286	0.0427

TABLE 9

SULPHUR FRACTIONS (GRAMS PER 100 PLANTS)

Solution	Volatile S	S in extract	S in residue	Total S
Minus-S....	0.0007	0.0335	0.0000	0.0342
Minus-S....	0.0013	0.0189	0.0000	0.0202
Plus-S.....	0.0199	0.2673	0.0147	0.3020

Discussion

In the previous papers (2, 3), rather detailed discussions have been given of the effects of sulphur deficiency on the appearance and chemical composition of the plant, the apparent explanation and interrelation of these effects, and their relation to the effects of the deficiencies of other elements. Since the present work on the mustard largely confirms the previous studies, these discussions will not be repeated here. Instead, a general review follows (*a*) summarizing the influence of sulphur deficiency on plants in general, (*b*) describing in what respects the effects differ in various plants, and (*c*) discussing the relation of sulphur deficiency to the volatile sulphur content of the mustard.

Summary of effects of sulphur deficiency on plants

The main symptoms of sulphur deficiency are the yellow-green color of the leaves, the smaller leaves, and the thinner stems as compared with plants grown

with complete nutrient. The stems are usually hard and woody, and they usually have a low water content. Minus-sulphur plants generally exhibit great power of stem elongation. Flower and fruit development are likely to be early.

Sulphur deficiency also results in the accumulation in the plant of the soluble organic nitrogenous compounds such as amino acids and amides; the inorganic nitrogenous compounds, ammonia and nitrates; and the polysaccharides, especially starch. The plant is also high in the soluble organic sulphur compounds, although these have not been studied very completely in this series of studies. The sugars, especially the reducing sugars, are usually low as compared with plus-sulphur plants.

Because of the great accumulation in minus-sulphur plants of the soluble organic sulphur and nitrogenous compounds, an abnormal amount of the latter being amides, because of the reduction in sugars of these plants, and because of their great power of stem elongation, these data have been interpreted as indicating proteolysis and the reutilization of the resulting sulphur and nitrogenous compounds in stem elongation. Sulphur deficiency has been found to reduce reductase activity (6). In this way the accumulation of nitrates may be accounted for, and the resulting decrease in protein synthesis may account for the accumulation of starch. Poor nitrate utilization would seem to account for the reduced chlorophyll development, and the hardness of the stems is probably to be explained by their high content of starch and to a less extent of acid-hydrolyzable carbohydrates.

If this is the correct explanation, it is a rather unique mechanism that enables the plant to carry on the processes of proteolysis and starch accumulation at the same time. Proteolysis is usually accompanied by a decrease in all forms of carbohydrates; slow nitrate reduction, by their accumulation. In a sulphur-deficient plant reducing sugars are usually low but starch is high. A minus-sulphur plant is in many respects similar to a minus-nitrogen plant, but the latter accumulates all kinds of carbohydrates because of poor protein synthesis resulting from the deficiency of nitrates. There is little proteolysis; most of the nitrogen is in the form of protein rather than of soluble compounds. There is limited power of stem elongation.

In the previous studies (2, 3), reference has been made to another method of explaining, in part at least, the accumulation in a sulphur-deficient plant of soluble organic nitrogenous compounds. All plant proteins apparently contain sulphur. The species specificity of proteins is a well-established concept. Calculations comparing the organic nitrogen of seed and plant show that a minus-sulphur plant synthesizes considerable organic nitrogen. But owing to the great deficiency of the sulphur-containing amino acids, condensation of the nonsulphur-containing amino acids and the amides to the proteins characteristic of the species is largely prevented; hence they accumulate.

But certain features can hardly be accounted for by reduced protein synthesis of a sulphur-deficient plant. Among these are the accumulation of soluble organic sulphur compounds, the great reduction in reducing sugars, and the power of elongation. It would seem that proteolysis is going on in these plants more rapidly than in the plus-sulphur ones, and that this accounts—in part at least—for the much larger amounts of the soluble organic nitrogenous and sulphur compounds. The rather large proportion of amides to amino acids in the nitrogenous material would also seem to be in line

with this conclusion, although this may perhaps be accounted for in other ways. It is likely, therefore, that both proteolysis and reduced protein synthesis are factors in the accumulation in the sulphur-deficient plant of soluble organic sulphur and nitrogenous compounds.

Differences in effects of sulphur deficiency on plants

While plants are rather uniform in the nature of sulphur-deficiency symptoms, they differ in the speed with which these symptoms develop. The sunflower (3) early showed both size and color differences. The difference in size in the mustard was soon evident, but chlorosis developed late. The effects of sulphur deficiency on both size and color developed late in the soybean (2). Nightingale (9) found that the symptoms developed gradually in the tomato.

Such differences are no doubt to be expected. The sulphur requirements of plants vary. Also the amount of sulphur introduced as an impurity in the various experiments was no doubt different. Another paper (4) gives the main sources of sulphur as an impurity. These are the sulphur in the seed or plant used, the sulphur in the air, and the sulphur in the salts of the nutrient solution. For plants grown in the open the sulphur in rainwater constitutes another source.

Plants vary in both the nature of the chlorosis and its gradation. Usually the chlorosis is uniform over the entire leaf, but in the case of the tea bush (14) the leaves become mottled. As to the gradation, the upper leaves generally show the chlorosis first. This is true of soybean (2), mustard, tobacco (8), and apparently of the tea bush (14). No gradation was evident in the sunflower (3), and Nightingale (9) found that the lower leaves of sulphur-deficient tomato plants become yellow-green first.

Increased cell-wall thickness is usually a prominent effect of sulphur deficiency. Nightingale (9) emphasizes it in the tomato. The stems were not examined anatomically in this series of studies, but it was noticed that the minus-sulphur soybean (2) and mustard stems were harder than the plus-sulphur ones. This did not seem true of the sunflower (3). Often the stiff texture of minus-sulphur leaves is prominent. This was true of the mustard, and Storey and Leach (14) describe it as a prominent symptom in the tea bush. This was not evident in the soybean and the sunflower.

Stem elongation is generally a prominent feature of sulphur-deficient plants, but the degree of prominence varies. Nightingale (9) found that the minus-sulphur tomato plants were as tall as or taller than the plus-sulphur. The sulphur-deficient soybean (2) and sunflower (3) plants elongated remarkably but were decidedly shorter on the average than the plants grown with complete nutrient. Stem elongation was not marked in the mustard until shortly before flower-bud formation. Since the plant naturally elongates at this time, it is impossible to separate the effects of sulphur deficiency and the reproductive processes.

The usual situation in regard to the chemical composition of sulphur-deficient plants has been given in a previous paragraph. The main variation from this is that, while generally the reducing sugars are low in these plants as compared with those grown with complete nutrient, these sugars accumulate in the stems of the tomato (9) together with the other carbohydrates. The relative amount of sucrose also varies in different plants. Minus-sulphur tomato and mustard stems are high in sucrose as compared with plus-sulphur stems; on the other hand, minus-sulphur sunflower (3) and

soybean (2) stems are relatively low in this sugar.

It is rather hard to account for these differences in the carbohydrate content of sulphur-deficient plants. It may be due to a variation in the relative influence of proteolysis and lessened reductase activity, both of which are phenomena of plants grown with a deficiency of sulphur. Proteolysis is usually preceded or accompanied by a reduction in the amounts of the carbohydrates present; interference with reductase activity results in the accumulation of carbohydrates owing to poor protein synthesis. On this basis the influence of the lessened reductase activity would seem to be predominant in the tomato, causing the accumulation of all forms of carbohydrates. But in the other plants studied, proteolysis is accompanied by the usual reduction in carbohydrates, at least to the extent that the reducing sugars are low, interference with reductase activity operating to cause the accumulation of starch and usually also to a certain extent of the acid-hydrolyzable carbohydrates. In regard to sucrose, sometimes the latter influence operates and it accumulates; sometimes the former, and its content decreases.

Influence of sulphur deficiency on volatile sulphur content

Of the three plants studied in this investigation, the mustard is the only one containing an appreciable amount of volatile sulphur. Tests indicated that the sunflower (3) and the soybean (2) contained little if any. But since in a previous paper (5) considerable data have been given as to the amount of volatile sulphur in the organs of the black mustard and the effects of sulphur deficiency and other conditions on the amount present, the subject will be discussed only briefly here.

The volatile sulphur compound of the seed, and presumably of the plant, is allyl mustard oil, $CH_2CHCH_2 \cdot NCS$. The leaves contain more of the compound than the stems and the young leaves more than the older leaves. Growing the plant in a nutrient solution lacking sulphur results in the almost complete lack of development of volatile sulphur. The leaves taste flat; they do not have the sharp, acrid taste characteristic of mustard, and analyses show that very little volatile sulphur is present. The present work confirms this as regards the stems (table 8). No work was done on the leaves.

Both nitrogen and sulphur are components of allyl mustard oil. It might perhaps be expected that growing the plants in solutions lacking these elements would result in decreased pungency. But while minus-sulphur leaves contain very little volatile sulphur (5), and the volatile sulphur content of plant tissue varies directly with the sulphur content of the growth medium (13), minus-nitrogen leaves are even more pungent than plus-nitrogen ones (5). The nature of the regulatory mechanism causing the difference is not known. However, with this difference in the relationship of sulphur deficiency and nitrogen deficiency to pungency, it is hardly surprising that minus-nitrogen leaves contain more volatile sulphur than plus-nitrogen ones. The sulphur content of the nutrient solution in which the two sets of plants grew was the same. The former leaves were much smaller than the latter. They had available, therefore, much more sulphur per unit of tissue.

Summary

1. The effects of sulphur deficiency on the size of black mustard plants was evident early, but chlorosis of the leaves appeared late. The main symptoms were the short plants, the yellow-green color of the upper leaves, the small leaves and the

thin stems. The stems were hard and the leaves had a stiff texture. Anthocyanin development in leaves and stems, the hairy nature of these organs, and the pimply character of the young leaves were prominent. The weight of the tops was reduced more than that of the roots, causing a low top-root ratio; but of the tops the stems were affected more than the leaves. Stem elongation was not prominent.

2. The sulphur-deficient stems were low in moisture. They were very high in ammonia, amino acids, and amides. Nitrates accumulated to a certain extent. The minus-sulphur stems were very low in reducing sugars, but they were much higher in sucrose and starch and also somewhat higher in acid-hydrolyzable carbohydrates than the stems of the plus-sulphur plants. Little volatile sulphur was found in the sulphur-deficient stems.

3. The chemical composition of the sulphur-deficient stems seemed to be due mainly to the combined influences of proteolysis and poor reductase activity. The breakdown of proteins accounted mainly for the accumulation of ammonia, amides, and amino acids, although this was no doubt partly the result of reduced protein synthesis owing to deficiency of sulphur-containing amino acids. Slow reduction of nitrates caused their accumulation and the resulting poor nitrate utilization for the accumulation of sucrose, starch, and acid-hydrolyzable carbohydrates. The accumulation of these carbohydrates accounted for the hard stems and stiff texture of the leaves. Proteolysis is usually accompanied by decrease in the carbohydrates. This was true in the present work to the extent that the reducing sugars were low in the minus-sulphur stems.

4. The effects of sulphur deficiency on plants in general are summarized and compared.

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LITERATURE CITED

1. DAVIDSON, O. W., and SHIVE, J. W., Determination of the nitrogenous fractions in vegetative tissue of the peach. Plant Physiol. **10**:73–92. 1935.
2. EATON, S. V., Influence of sulphur deficiency on the metabolism of the soybean. BOT. GAZ. **97**:68–100. 1935.
3. ———, Influence of sulphur deficiency on metabolism of the sunflower. BOT. GAZ. **102**: 536–556. 1941.
4. ———, Sulphur content of seed and seed weight in relation to effects of sulphur deficiency on growth of sunflower plants. Plant Physiol. **17**: 422–434. 1942.
5. ———, Volatile sulphur content of black mustard plants. BOT. GAZ. **104**:82–89. 1942.
6. ECKERSON, SOPHIA H., Conditions affecting nitrate reduction in plants. Contrib. Boyce Thompson Inst. **3**:197–217. 1931.
7. LIVINGSTON, B. E. (Editor), A plan for cooperative research on the salt requirements of representative agricultural plants. 2d ed. Baltimore. 1919.
8. MCMURTREY, J. E., Symptoms on field-grown tobacco characteristic of the deficient supply of each of several essential chemical elements. U.S.D.A. Tech. Bull. 612. 1938.
9. NIGHTINGALE, G. T., SCHERMERHORN, L. G., and ROBBINS, W. R., Effects of sulphur deficiency on metabolism in tomato. Plant Physiol. **7**:565–595. 1932.
10. PHILLIPS, T. G., *et al.*, The determination of nitrogen in relatively simple compounds. Plant Physiol. **2**:205–211. 1927.
11. ———, The determination of nitrogen in relatively simple compounds. First supplemental report. Plant Physiol. **10**:393–399. 1935.
12. PLATENIUS, HANS, A method of estimating the volatile sulphur content and pungency of onions. Jour. Agr. Res. **51**:847–853. 1935.
13. PLATENIUS, HANS, and KNOTT, J. E., Factors affecting onion pungency. Jour. Agr. Res. **62**: 371–379. 1941.
14. STOREY, H. H., and LEACH, R., A sulphur deficiency disease of the tea bush. Ann. Appl. Biol. **20**:23–56. 1933.
15. STUART, N. W., Determination of amino nitrogen in plant extracts. Plant Physiol. **10**:135–148. 1935.
16. SULLIVAN, J. T., Report on carbohydrates in plants. Study of the permanganate titration for copper. Jour. Assoc. Off. Agr. Chemists **18**: 382–386. 1935.
17. WEBSTER, J. E., Nitrogen changes in stored alcoholic extracts of plant tissue. Plant Physiol. **8**:166–168. 1933.

NUTRITIONAL EFFECTS OF BORON ON GROWTH AND DEVELOPMENT OF THE SUNFLOWER

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 550

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Introduction

Although a number of possible roles of boron in the nutrition of plants have been suggested, sufficient data are not available to lend more than tentative support to any of them. SKOLNIK (15) and REHM (11) indicate that boron serves to regulate the accumulation of ions from the nutrient substrate. WARINGTON'S (20) results suggest that boron is especially correlated with the intake of calcium. That such intake is lessened in the absence of boron has been corroborated with the soybean (9). SHIVE (14) found, in maize and in the broad bean, that—in the absence of boron—the total calcium in the plant was not detectably reduced, but that the soluble calcium was much less.

SMIRNOV (16) concluded that boron acts to regulate the normal growth of the various parts of the tobacco plant, chiefly the stem. The influence of boron on the growth of roots and leaves is less marked than on the other parts of this plant. The effect of boron deficiency on the vascular system suggests a relationship between boron and translocation of the nutrient materials. MORRIS (10) uses this to explain the fact that citrus plants grown under boron deficiency showed proportions of organic and inorganic materials in the leaves and fruits different from the proportions in plants to which boron was supplied.

To date, the influence of boron on the accumulation of materials from the nutrient solution has been studied to a considerable extent by means of analyses of the nutrient medium from which the roots of the plants absorb the materials. A direct analysis of the plant itself has many advantages, however, especially if it can be divided into parts before analysis, so that local accumulation can be ascertained. It was with this in mind that the present work was undertaken.

Methods

The methods used in growing the plants were essentially those employed by EATON (3). On May 4, 1941, sunflower seedlings (*Helianthus annus* L., variety Mammoth Russian) 3 days old were transplanted to quartz sand in glazed crocks of about 1250-cc. capacity. SHIVE'S (13) three-salt solution was used, except that ferric citrate instead of ferric tartrate was employed. Only c.p. and analytical-grade chemicals were used. When the plants were well established, they were thinned to one per crock, leaving in all fifty plants.

On May 24, when the plants were 23 days old, differential treatment was started. The plants were divided into fourteen groups of three plants each and the others discarded. The groups were paired in such a way that the members of each were as nearly similar to the members of the adjacent group as possible.

The sand of all crocks was rinsed thoroughly with distilled water before the

plus- and minus-boron treatment was begun. Half the plants received nutrient solution containing added boron at a concentration of 0.75 p.p.m.; the other half had only such boron as may have occurred as impurities.

The plants were harvested 6 days after starting the treatment. They were divided into (1) stem tips, (2) leaves and petioles, (3) stems, and (4) roots. First the stem tips of a minus-boron sample were removed, selecting the approximate level where blackening of the stem tissue appeared. The tips of the stems of the corresponding plus-boron plants were then harvested, the cut being made at the point on the stem closely corresponding to the place where the minus-boron stems were cut. Since the plus-boron stems had elongated much more than the others, it was not possible to estimate this point accurately. In every case the cut was made in the fifth internode above the cotyledons.

The leaves and petioles were harvested and all above-sand portions of the plant weighed. Most of the sand was removed from the roots by rinsing with salt water, followed by thorough rinsing—first with tap and then with distilled water. All the parts were dried to constant weight at 90° C. and reweighed. The sand left on the roots was largely removed after drying by blowing the dry material. The samples were ashed in platinum crucibles in a muffle furnace at 600° C. The sand left on the roots after this treatment was determined as the hydrochloric acid-insoluble portion of the ash.

Calcium was determined in an aliquot of the ash by means of the Official Methods (1). MINARIK and SHIVE (9) have pointed out that precipitation of the silicon dioxide is not necessary, and accordingly this step was omitted.

The method of statistical analysis as described by SNEDECOR (17) was used in determining the significance of the data. Although the plants were arranged in paired replications as described, most of the analyses were made as pooled data.

Results

The plants were entirely healthy in appearance during the entire growing period before treatment was started. Twenty-four hours after placing the plants on minus-boron treatment, they showed the first indications of injury. This consisted of a pubescent grey appearance of the terminal buds and newly expanding leaves. No stem elongation occurred in these plants from the time minus-boron treatment was started. On the day of harvesting, the younger leaves were curled downward, the base was lighter green than the tip, and the tissue had become brittle. Black longitudinal streaks were visible at the time the tip of the stem was cut. Microscopic examination of similar plants indicated that these were the vascular bundles which had failed to differentiate normally.

Table 1 summarizes the data. The fresh weights of all the above-ground parts of the plant, except the leaves, were heavier in the case of the plus-boron than in the case of the minus-boron plants. No significant difference in the fresh weight of the leaves of the two groups is indicated.

The plus-boron plants contained a higher percentage of moisture. The minus-boron plants tended to become flaccid in warm clear weather, apparently not absorbing sufficient moisture to keep up with transpiration. The morning of May 30, when harvesting was done, was cool and cloudy, however, and the minus-boron plants were turgid.

The percentage of ash in the minus-boron plants was lower in the stem tip

TABLE 1

EFFECTS OF BORON SUPPLY ON SUNFLOWER PLANT

Data	Plant part															
	Stem tip			Stem			Leaves			Root			Total			
	Treatment		Difference	Treatment		Difference	Treatment		Difference	Treatment		Difference	Treatment		Difference	
	+B	−B		+B	−B		+B	−B		+B	−B		+B	−B		
Fresh weight (gm.)	25.00	16.04	8.96±1.56	37.94	27.09	10.85±1.97	15.7	16.30	−0.6 ±1.0	.....	.....	.....	78.31*	59.79	18.52±2.86	
Moisture (%)	90.57	89.61	0.96±0.15	94.30	92.95	1.35±0.10	91.96	91.10	0.86±0.19	.....	.....	.....	92.67*	91.50	1.17±0.11	
Dry weight (gm.)	2.34	1.67	0.68±0.14	2.16	1.92	0.24±0.13	1.27	1.46	−0.19±0.10†	1.39	1.17	0.22±0.05	7.13	6.23	0.91±0.24	
Ash (%)	16.36	14.53	1.83±0.27	16.7	17.00	−0.3 ±0.4	21.47	21.66	−0.19±0.65	22.7	21.4	1.3 ± 0.9	18.78	18.28	0.50±0.16	
Total ash per plant (gm.)	0.38	0.25	0.14±0.04	0.35	0.32	0.03±0.03	0.27	0.32	−0.05±0.02†	0.30	0.25	0.05±0.02	1.31	1.16	0.15±0.04	
Total combustible material per plant (gm.)	1.96	1.45	0.51±0.17	1.74	1.59	0.14±0.15	1.01	1.25	−0.24±0.09†	1.03	0.91	0.12±0.05	5.66	5.26	0.40±0.20	
Calcium in ash (%)	11.73	11.47	0.26±0.22	5.28	4.68	0.60±0.18	13.30	14.00	−0.72±0.26†	9.32	6.60	2.7 ±0.43	9.81	9.22	0.59±0.18	
Total calcium per plant (mg.)	45.30	28.40	16.9 ±4.80	18.40	15.20	3.20±1.83	36.18	45.27	−9.1 ±2.83†	28.82	16.70	12.1 ±1.64	128.94	109.67	19.3 ±6.70	

* Value does not include root. † Data paired in analysis.

and total plant. The total ash in the roots, stem tip, and entire plant was lower in the case of the minus-boron plants but significantly higher in the leaves.

The percentage of calcium in the ash of the minus-boron stems and roots was significantly lower than in the plus-boron. The reverse was true for the leaves, the plus-boron ones containing a lower percentage of calcium than the minus-boron. The percentage of calcium in the entire plant showed a significant difference in favor of the plus-boron group. The stem tip and root of the minus-boron plants contained less calcium, while the leaves contained more.

The total combustible material was determined as the difference between the dry weight of the material and the weight of ash (table 1). The data show that the weight of material lost in ashing the stem tip and root was greater in the plus-boron plants. On the other hand, the weight of material lost in ashing the plus-boron leaves was significantly less.

Plants grown under conditions similar to those under which the plants used in analysis were grown, and which were then transferred to treatment with distilled water, showed injury to the terminal portions of the stems. These injuries could not be distinguished from minus-boron symptoms, except that they progressed much more slowly. A few days after the symptoms of the stem tip appeared, the lower leaves of these plants became yellow. These eventually withered, and the yellowing and gradual death progressed toward the higher leaves. This symptom was not displayed by the minus-boron plants. Treating the plants which had been on distilled water with a solution containing 0.5 p.p.m. of added boron did not appear to affect the lower leaves, but recovery of the stem tip took place. This recovery was similar to that of minus-boron plants when transferred to complete nutrient solution, except that it was not so rapid.

Discussion

While the plus-boron plants remained completely healthy in appearance during the entire growth period, the minus-boron plants developed distinct symptoms of injury upon their transfer to minus-boron conditions. These symptoms consisted of total cessation of growth of the stem tip and change to the characteristic grey color of the young leaves. The appearance of the discolored and misshapen leaves and stem tips of the plants suffering from boron deficiency is distinctive. SCOFIELD, WILCOX, and BLAIR (12) illustrate the upper portion of a sunflower plant showing characteristic minus-boron symptoms.

Considerable significance may be attached to the observation of the plants transferred to distilled water. SOMMER and SOROKIN (18) found that in general the absence of boron and of certain other elements caused symptoms similar to those induced by the absence of boron alone. The present results suggest that boron is the first of all nutrient elements to become deficient, and that to this extent it limits the utilization of all other nutrients. The rapid appearance of minus-boron symptoms in plants having the other essential elements now acquires further significance.

The percentage of water in the tissues of the two lots does not agree with the findings of MINARIK and SHIVE (9). They found that when soybean plants were grown from seed under different levels of boron, the plants higher in percentage of boron were lower in moisture. While at first the present results seem to conflict with their data, the probable explanation is that their plants were de-

veloped under an almost unchanging boron level throughout life. In the present case the plants were grown under an adequate boron supply and then transferred to a deficient medium.

The data at hand support three generalizations.

1. EFFECT OF BORON DEFICIENCY ON WEIGHT INCREASE.—The reduction in growth of root and stem tip is striking. It is in agreement with the findings of JOHNSTON and DORE (7) but does not substantiate SMIRNOV'S (16) assertion that the influence of boron on the growth of the tobacco root is not marked. The length of the stem was considerably greater in the plus-boron plants, for it had elongated during the time that the minus-boron plants showed no growth. This is reflected in the fresh weight of the plus- and minus-boron stems. Apparently this elongation was accomplished largely by means of vacuolization of the cells, without considerable increase in solids, for the dry weights do not differ significantly. The data indicate that the parameter of the dry weight of the minus-boron leaves is at least as great as, and probably greater than, that of the plus-boron leaves.

2. PERCENTAGE ASH AND COMBUSTIBLE MATERIAL.—The evidence indicates that the percentage of ash in the stem tip was lowered by the removal of boron, and that the percentage of combustible material was accordingly increased. No other differences in the ash of the various parts are shown, but the average for the entire plant shows a significant difference in favor of the plus-boron plants. It is possible that had the plants been grown in water culture, a difference in the percentage of ash of the roots could have been determined. The sand clinging to the roots constituted an interfering factor which was never completely eliminated. HILL and GRANT (6) state that in turnip the roots of high-boron plants are lower in ash than are those with boron deficiency. The total ash and combustible material is a correlative of the dry weight and the percentage of ash of the plant parts. The data indicate that even though the percentage of ash in the stem tips of the minus-boron plants is less than in the plus-boron plants, the total of material lost in ashing (as well as of ash) is less. This is caused by the fact that the total weight of the stem tips of the minus-boron plants is considerably less than of the plus-boron plants. In the roots also, the minus-boron plants are lower in ash and in combustible material, but the reverse seems to be the case in the leaves. It seems improbable that this is caused by the removal to the leaves of materials from the roots and stem tips, for most of the plant nutrients are fixed in a form not readily transportable. The only other explanation, assuming that the differences between the plus- and minus-boron leaves are not caused entirely by chance, is that after the plants were transferred to minus-boron solution the net accumulation of inorganic ions from the nutrient medium was hastened in the leaf and the loss of phytosynthates reduced.

3. AMOUNT OF CALCIUM IN THE ASH.—No differences in the percentage of calcium in the ash of the stem tips could be observed. The ash of the minus-boron leaves was higher in calcium than that of the plus-boron leaves, while in the stem, root, and total plant it was significantly lower. The fact that no differences in the percentage of calcium in the stem tips of the plants could be observed indicates that boron does not have a direct effect upon calcium utilization by the cells of this part of the plant. Other workers have not observed the difference in calcium in the total plant, but since it

is small it could be detected only by controlled replications. MARSH and SHIVE (8) and SHIVE (14) report that in maize and in the broad bean the availability of a boron supply is not correlated with the amount of calcium in the plant, but that there is a strong correlation between boron and soluble calcium.

Apparently boron is essential for the normal distribution of materials through the various parts of the plant. In the absence of boron, the materials ordinarily used in the growth of the root and stem tip accumulate in the leaf. Furthermore, as has been observed by others, boron is essential for the formation and maintenance of the vascular system. CHANDLER (2) concluded, after observation of boron deficiency in a number of species in the Cruciferae, that boron is intimately associated with differentiation of cells. HAAS and KLOTZ (5) have observed in citrus, ELTINGE (4) in maize, and WARINGTON (19) in the broad bean, that the vascular system is not normal in plants deprived of boron. In the present experiment the phloem of the minus-boron plants appeared to be filled with a gum-like substance, in both the younger and older portions of the stem. No deterioration of the xylem was observed in the mature stem, but the secondary xylem failed to differentiate, apparently remaining parenchymatous. In the young stem most of the xylem region was devoid of tracheae. These facts, together with the data for the weights of the different parts of the plant, strongly indicate that boron is directly necessary for the maintenance of the vascular system, and that, in the absence of boron, materials cannot pass freely from one part of the plant to another. The data here presented constitute direct supporting evidence to this theory, previously suggested by others.

SCOFIELD, WILCOX, and BLAIR (12) suggest the use of indicator plants for determining whether the crop production of a field will be increased by boron fertilization. They suggest that the amount of boron in the leaf of a sunflower plant grown under standard conditions be used as the index of boron deficiency, but the method is unwieldy and probably not entirely accurate. The data in the present experiment suggest that the interrelationship between boron availability and the weight of the various portions of the plant is of practical value in determining when boron fertilization is desirable. The average quotient leaves/remainder of plant was 0.220 for the plus- and 0.308 for the minus-boron plants. This relationship may not hold under field conditions, or be sufficiently constant for use as a measure of boron deficiency, but the data suggest that the possibility is worth consideration.

Summary

1. Sunflower plants showed typical boron-deficiency symptoms promptly upon transfer from complete nutrient solution to one deficient in boron.

2. Plants transferred from complete nutrient solution to distilled water developed symptoms typical of boron deficiency. Upon supplying these with a dilute solution of boric acid, 0.5 p.p.m. of boron, partial recovery occurred.

3. Boron was found necessary for growth of the stem tips and roots. Inconclusive evidence was obtained that the leaves of plants were heavier if the plants were deprived of boron during the later part of the growth period.

4. The stem tips of the minus-boron plants contained a smaller percentage of ash. Elsewhere in the plant, differences in percentage of ash could not be observed.

5. No difference in the percentage calcium in the ash of the stem tips of the plus- and minus-boron plants could be observed. The ash of the leaves of the minus-boron plants contained more calcium, and the ash of the stem, roots, and entire plant contained less.

6. Available evidence supports the hypothesis that boron, because of its importance in the maintenance of the vascular system, is instrumental in regulating the distribution of materials through the various parts of the plant. The calcium determinations are in accord with the view that there is a relationship between boron availability to the plant and the utilization of calcium.

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LITERATURE CITED

1. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and tentative methods of analysis. 5th ed. 1940.
2. CHANDLER, F. B., Mineral nutrition of the genus *Brassica* with particular reference to boron. Maine Agr. Exp. Sta. Bull. 404. 1941.
3. EATON, S. V., Effects of boron deficiency and excess on plants. Plant Physiol. 15:95–107. 1940.
4. ELTINGE, E. T., Effect of boron deficiency upon the structure of *Zea mays*. Plant Physiol. 11: 765–778. 1936.
5. HAAS, A. R. C., and KLOTZ, L. J., Some anatomical and physiological changes in citrus produced by boron deficiency. Hilgardia 5:175–196. 1931.
6. HILL, H., and GRANT, E. P., The growth of turnips in artificial cultures. Sci. Agr. 15:652–659. 1935.
7. JOHNSTON, E. S., and DORE, W. H., The influence of boron on the chemical composition and growth of the tomato plant. Plant Physiol. 4: 31–62. 1929.
8. MARSH, R. W., and SHIVE, J. W., Boron as a factor in the calcium metabolism of the corn plant. Soil Sci. 51:141–151. 1941.
9. MINARIK, C. E., and SHIVE, J. W., The effect of boron in the substrate on calcium accumulation by soybean plants. Amer. Jour. Bot. 26:827–831. 1939.
10. MORRIS, A. A., Some observations on the effects of boron treatment in the control of "hard fruit" in citrus. Jour. Pomol. Hort. Sci. 16:167–181. 1938.
11. REHM, SIGMUND, Der Einfluss der Borsäure auf Wachstum und Salzaufnahme von *Impatiens balsamina*. Jahrb. wiss. Bot. 85:788–814. 1935.
12. SCOFIELD, C. S., WILCOX, L. V., and BLAIR, G. Y., Boron absorption by sunflower seedlings. Jour. Agr. Res. 61:41–56. 1940.
13. SHIVE, J. W., The adequacy of the boron and manganese content of natural nitrate of soda to support plant growth in sand culture. New Jersey Agr. Exp. Sta. Bull. 603. 1936.
14. ———, Significant roles of trace elements in the nutrition of plants. Plant Physiol. 16:435–445. 1941.
15. SKOLNIK, M., On the physiological role of boron. Compt. Rend. Acad. Sci. L'urss 1:143–146. 1934.
16. SMIRNOV, A. I., Boron as regulator of tobacco growth in connection with the source of nitrogen and pH of nutrient solution. State Institute for Tobacco Investigations. Krasnodar. Bull. 70. 1930. (Russian with English summary.)
17. SNEDECOR, G. W., Statistical methods applied to experiments in agriculture and biology. Collegiate Press. Ames, Iowa. 1938.
18. SOMMER, A. L., and SOROKIN, H., Effects of the absence of boron and of some other essential elements on the cell and tissue structure of the root tips of *Pisum sativum*. Plant Physiol. 3: 237–260. 1928.
19. WARINGTON, K., The changes induced in the anatomical structure of *Vicia faba* by the absence of boron from the nutrient solution. Ann. Bot. 40:27–42. 1926.
20. ———, Studies in the absorption of calcium from nutrient solutions with special reference to the presence of absence of boron. Ann. Bot. 48:743–776. 1934.

DIFFERENTIATION OF AVOCADO BLOSSOM BUDS IN FLORIDA

PHILIP C. REECE[1]

(WITH THIRTEEN FIGURES)

Many species of cultivated deciduous fruit trees have been studied to determine the time of floral initiation, but less is known regarding the time of fruit-bud differentiation in evergreen tropical and subtropical fruit trees. Such knowledge is important because of the possibility of influencing differentiation by cultural practices.

Most deciduous fruit trees (for example, apple, pear, plum, and cherry) initiate flowers during the summer following their period of vegetative growth in the spring. Most of the floral organs are formed before the leaves fall and emerge the next spring after a period of dormancy. Fruit trees of the tropics and subtropics, which usually exhibit several distinct cycles of vegetative growth within the season, are not in such a category. Frequently it is difficult to distinguish between a flower bud and a purely vegetative one before it opens, either from its size or position. ABBOT[2] has shown that blossom-bud differentiation takes place in citrus during initiation of growth in the spring or upon resumption of growth at any other season as the result of a period of favorable environmental conditions. Flowers may thus appear on axes which did not exist a few months earlier.

Terminal and lateral buds of the avocado were collected at monthly intervals, beginning October 8, 1940, from trees of the Lula and Nabal varieties, growing on the grounds of the United States Horticultural Field Laboratory at Orlando, Florida. Some variation in the stage of development existed over various parts of a tree, so that each collection was taken from branches which represented as nearly as possible the average stage of bud development at the time of collection. From these branches the terminal buds and the four to six lateral buds nearest the terminal portion were taken for sectioning. The buds were killed in 70 per cent alcohol, imbedded in paraffin, and sectioned longitudinally at 10–15 μ.

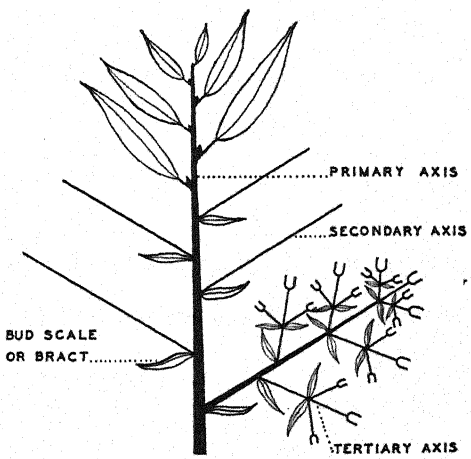


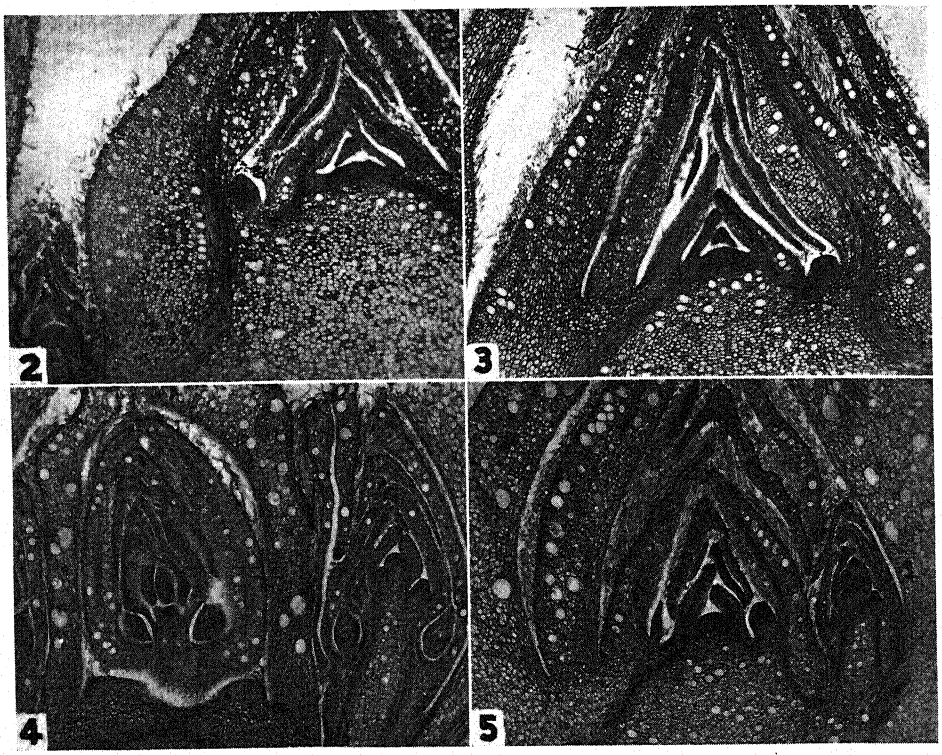
FIG. 1.—Diagram of floral and vegetative growth developed from terminal or lateral winter buds of avocado.

At first, as the terminal and lateral winter buds unfold, growth appears to consist of inflorescences only. Each in-

[1] Junior Botanist, Division of Fruit and Vegetable Crops and Diseases, U.S. Department of Agriculture.

[2] ABBOT, C. E., Blossom-bud differentiation in citrus trees. Amer. Jour. Bot. 22:476–485. 1935.

florescence seems several times compound. Later it becomes evident that each main axis is an indeterminate branch. The first leaves formed on this axis are bractlike, and each subtends an axillary bud which develops into an inflorescence (fig. 1). These lateral inflorescences or secondary axes are alternately arranged on the primary indeterminate axis of the winter bud and develop acropetally, giving the whole group the appearance of a compound raceme or panicle. Subsequent development shows that actually the secondary axes constitute the inflorescences. On these axes the terminal or central flowers mature first, and the clusters are therefore cymes. The growing point of the primary axis extends the vegetative growth of the shoot beyond the region of the diverging inflorescences. Foliage leaves are formed, and the buds in their axils are vegetative.



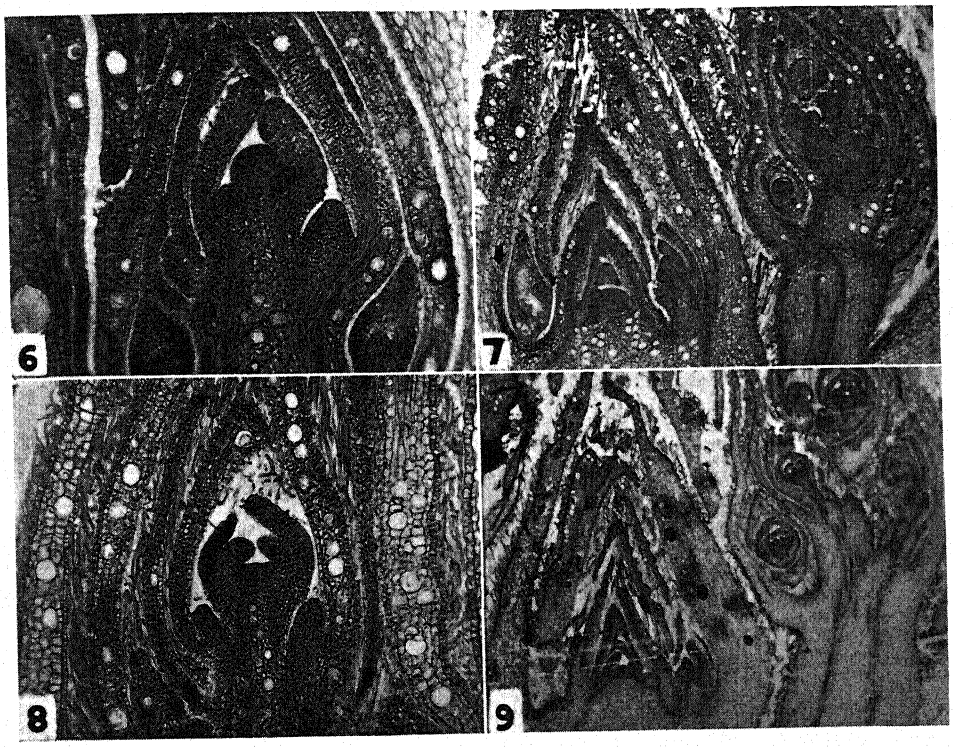
FIGS. 2–5.—Fig. 2, terminal bud of Lula variety on Oct. 8; bracts appear on lowest secondary axis. Fig. 3, terminal bud of Nabal variety on Oct. 8; meristems of secondary axes show no differentiation. Fig. 4, longisection of Nabal terminal bud, Dec. 10; secondary axes with bracts differentiated and meristems for tertiary axes apparent. (Section not truly median and therefore does not show primary apical meristem.) Fig. 5, Lula terminal bud on Dec. 10; lower secondary axis appears and differentiation is progressing. A higher secondary axis does not show differentiation. Apical meristem of primary axis appears in this section.

In mid-September most branches of the trees were actively vegetative. The first collection was made on October 8, 1940, when growth elongation had ceased and terminal buds had formed. The condition of terminal and lateral buds was found so similar in the case of each variety that this discussion will be

confined to terminal buds. In October meristematic areas were present in the axils of the bud scales. Occasionally the lowest meristem within the bud had differentiated several bracts (fig. 2); in other buds the meristems for secondary axes consisted of undifferentiated mounds of cells (fig. 3). One month later no change was recognizable in most of the buds of both varieties. One bud showed some elongation of the lowest secondary axis, but only bracts were differentiated on it. Meristematic areas in the axils of the distal bud scales were undifferentiated in all buds at this date.

From November 8 to December 10 the formation of the lower secondary axes progressed, and meristematic areas appeared in the axils of the bracts on the secondary axes (fig. 4). From the alternate arrangement of bracts and meristematic areas (fig. 6) it is obvious that this condition cannot be an early stage in the differentiation of a flower but is simply a secondary axis with bracts and axillary meristems. Whether or not a particular meristematic area would have given rise to a single flower or continued the axial ramification of the inflorescence cannot be determined. Prior to December 10 no flower primordia could be detected.

By January 13 the lateral branches



FIGS. 6–9.—Fig. 6, secondary axis of Nabal terminal bud on Dec. 10, 1940. Meristems of tertiary axes appear in axils of bracts, which are arranged alternately on secondary axis. Fig. 7, terminal bud of Lula variety of Jan. 13, 1941; lower lateral branches show early stages in flower development; higher and younger branches within bud are in various stages of development. Fig. 8, rudimentary flower terminating secondary axis in a Nabal bud, Jan. 13. Fig. 9, longisection of Lula bud collected Feb. 11; axes of orders and flowers in various stages of development.

within a terminal bud were in various stages of development, and the proximal lateral branches showed early stages in flower development (fig. 7). These branches, or inflorescences, ultimately become cymose, and the floral differentiation and anthesis of the cymules become basipetal, the middle flower opening first (fig. 1). In material collected at this time rudimentary sepals and petals were evident in the terminal flower on the secondary axis (fig. 8), and meristematic areas which would have given rise to tertiary axes appeared beneath it. A flower terminating a tertiary axis and a portion of an axis of a fourth order also appeared in this section.

Material collected February 11, 1941, showed highly complicated buds with many lateral axes of various orders and flowers in various stages of development (fig. 9). Even at this time, however, the younger distal secondary axes of the bud appeared only as meristematic mounds of cells. However, there were more well-developed axes upon the various radii than were present at progressively earlier dates.

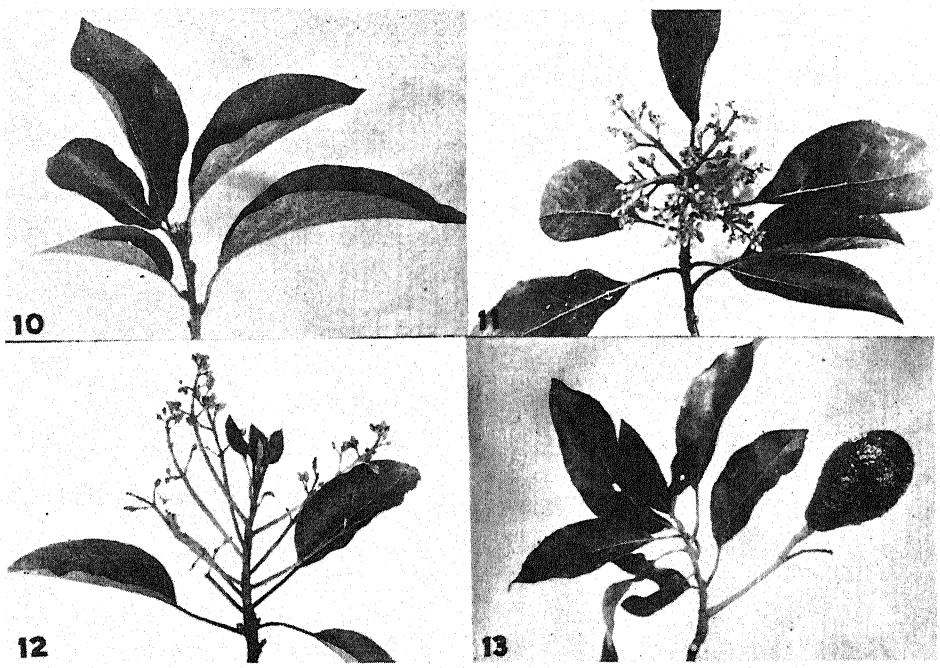
In order to compare the regional variation in the differentiation of the inflorescences of these varieties, as well as that from year to year, buds were collected bi-monthly from the trees at Orlando during the winter season of 1941–42 and monthly from trees of the same varieties on the grounds of Mrs. W. H. Krome at Homestead, Florida.

The inflorescences began their differentiation later during the winter of 1941–42 in the Orlando district than they did in 1940–41. Lula terminal buds collected December 31, 1941, were in approximately the condition shown in figure 5, which illustrates the stage reached by Lula buds at Orlando on December 10, 1940. In the Homestead region differentiation was even further delayed, and on January 14, 1942, Lula buds were still in the stage illustrated in this figure. Buds of both varieties collected the following day at Orlando showed early stages in the differentiation of flowers with the appearance of the first sepal primordia. Thus, during the period between December 31, 1941, and January 14, 1942, development took place approximately equal to that covered by the period from December 10, 1940, to January 13, 1941. Subsequent growth of the inflorescences in 1942 closely paralleled the development in the preceding year. At Orlando on February 10 Lula buds contained many flowers in which sporogenous tissue was present in the stamens, and ovule development had begun. Nabal buds were slightly less advanced and did not have sporogenous tissue. Although on January 14, 1942, buds of the Lula variety in the Homestead district were less advanced by 2 weeks than were buds at Orlando, and by a month in comparison with Orlando buds of the preceding year, by February 11 they equaled the development at Orlando. The same cannot be said of the development of the inflorescences of the Nabal avocado at Homestead. Buds collected on March 10 had developed little during the entire year. The lowest secondary axes appeared like those of the Lula variety on December 10, 1940 (fig. 5).

With the exception just mentioned, during early March the buds expanded rapidly (fig. 10), and the secondary branches from the bud underwent marked development. These secondary axes develop acropetally and give rise to tertiary branches which may either be alternate or apparently opposite in arrangement.

These tertiary branches have flowers in small clusters (cymules) in which the central blossoms opened first (fig. 11).

In the entire growth of the primary axis of the bud an indefinite number of secondary flowering axes appear and mature. Finally development of this nate condition of the primary axes. Although a group of inflorescences arising from a single winter bud may bear several hundred flowers, in most commercial varieties usually a single fruit matures, or—at best—a very small number. Thus most of the secondary axes or inflo-



FIGS. 10–13.—Fig. 10, terminal bud just before elongation of secondary axes. Fig. 11, inflorescences expanded and ultimate cymules visible. Fig. 12, flowers and various axes abscissing and vegetative growth of indeterminate primary axis developing. Fig. 13, fruit and its relation to secondary or inflorescence axis and to primary axis of winter bud.

type ceases. Nevertheless, the main apical meristem continues to grow and gives rise to the leafy or vegetative spring flush of growth (fig. 12). Each leaf on this upper portion of the main axis subtends an axillary bud which is purely vegetative in character. In a few abnormal cases the primary axis becomes determinate and a flower terminates the growth. Vegetative growth is therefore dependent upon the normally indeterminate rescences abscise. The primary axes continue vegetative growth, and the fruits that mature are usually borne upon axes of a third or fourth order (fig. 13).

Summary

1. Buds of Lula and Nabal avocado collected during the winters of 1940–41 and 1941–42 at Orlando, Florida, and from the same varieties at Homestead, Florida, during the winter of 1941–42,

were studied. In these varieties floral differentiation continued over a period of months during that part of the winter when leaf formation and growth did not take place or were at a minimum.

2. Differentiation began with development of the proximal secondary axes in late October or November. There was a year-to-year variation in the time of this development for a given variety, and between varieties, as well as between trees of the same variety in different sections.

3. The first identifiable flowers appeared on the lowest inflorescences (secondary axes) in the January buds of both varieties in the Orlando district and of the Lula variety in the Homestead district; but in the Nabal variety at Homestead they had not yet appeared on March 10, 1942. Differentiation proceeded distally during the winter, and in the most distal secondary axes it was completed just prior to or during expansion of the bud. Development into complete bloom was not interrupted by a dormant period between the time of initiation and differentiation on the inflorescence and its expansion in the spring.

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OPTIMAL CONDITIONS FOR THE HYDROLYSIS OF ARABOGALACTAN BY ASPERGILLUS NIGER

EDWIN J. RATAJAK AND HARRY S. OWENS

Introduction

GORTNER (16) states that, as far as is known, there is no specific enzyme by which the hydrolysis of galactans can be accomplished. The literature, however, indicates that such enzymes have been derived from various sources. BIERRY and GIAJA (3, 4) have found that the hepato-pancreatic juice of *Helix pomatia* attacks both mannans and galactans, and that the intestinal juice of *Astacus fluviatilis* sets free the galactose of the mannogalactan of alfalfa; the mannose, however, is only partially freed and in a degree is dependent upon the concentration of the enzyme. HERRISEY (17) has shown that certain species of *Aspergillus* are able to produce enzymes hydrolyzing mannans and galactans, when grown on solutions containing the respective carbohydrates. LUTZ (26, 27) has found that many Hymenomycetes secrete enzymes capable of hydrolyzing hemicelluloses and gums. He obtained the decomposition by enzymes of hemicelluloses—arabans, mannans, and galactans—which occur in many plants. In studying the aerobic decomposition of hemicelluloses, NORMAN (33) employed some seventy molds, mostly *Aspergilli* and *Penicillia*, and found that all the fungi tested grew reasonably well on media containing the crude hemicellulose from oat straw, which included 8.2 per cent uronic acid, 50.8 per cent pentose, and the remaining 41 per cent presumably hexosans. The majority of the molds grew at least as well as on glucose and a number better than on that sugar. In some cases there was a production of a cleared zone around the colony, due to an exo-enzyme. He believed it likely that the ability to ferment hemicelluloses will be demonstrated a general property of the common fungi. WAKSMAN and DIEHM (53) found that *A. niger* was able to decompose galactans to the extent of 37 per cent in 42 days but noted that the galactan was more resistant to decomposition by microörganisms than were mannans and xylans. The galactan used by these investigators was obtained from various sources, including straws, which were classified by them as hemicelluloses.

To study the action of a galactanase, if such exists, a source of the enzyme is required. Because *A. niger* seems able to produce a wide variety of enzymes, one of its strains was chosen for this study. Another requirement is a suitable galactan, which was supplied by arabogalactan.

Arabogalactan is a polysaccharide obtained from western larch (*Larix occidentalis*) in quantities varying from 10 to 20 per cent of the heartwood. It is also present to some extent in other species of larch and in southern pine. It can be extracted readily from sawdust by simple water leaching and consequently should undergo little chemical change during its isolation. It is fairly well characterized as a compound containing arabinose and galactose in the ratio of one molecule to six, respectively. Although the

exact arrangement of the sugar residues in the molecule is still unknown, information on that point is accumulating (56, 57, 58), so that before long not only the type and location of the linkages between the sugar residues, but also the spatial relationship between them, should be known. This substrate should offer possibilities for fundamental work on the action and specificity of many enzymes.

HOSSFELD (20) has investigated the action of several strains of *A. niger* on water extracts of larch sawdust, without obtaining conclusive results. Preliminary unpublished work by OWENS indicated that purified arabogalactan could be used as a satisfactory substrate for such molds. The optimal conditions for the maximum hydrolysis of arabogalactan by *A. niger* have now been established and are reported in this paper.

Material and methods

Arabogalactan was prepared in the same manner as reported earlier (34), by extracting sawdust from the heartwood of a western larch stump with water. The extract was decolorized with charcoal and the polysaccharide precipitated from solution by ethyl alcohol. The fraction obtained by addition of from one to four volumes of alcohol was used. The first fraction usually contained some colored impurities and inorganic matter. The remaining colored impurities were removed with charcoal. The fraction obtained with more than four volumes of alcohol was small in amount and was not economical to separate. Arabogalactan prepared in this manner analyzed 80–85 per cent anhydro-galactose and had an ash content of 0.1 per cent. The viscosity-concentration coefficient (10) was 5.8×10^{-3}, obtained by use of water solutions containing 1–4 per cent arabogalactan.

The strain of *Aspergillus niger* used was Thom 3528.7, obtained by Dr. John Ehrlich of the School of Forestry, University of Idaho, from Dr. Charles Thom of the Division of Soil Microbiology, Bureau of Plant Industry, United States Department of Agriculture.

All the chemicals in the media were c.p. and were used without further purification. The basal nutrient was made up within the limits proposed by CURRIE (7) and contained 1.0 gm. KH_2PO_4, 0.15 gm. $MgSO_4$, and 2.5 gm. NH_4NO_3 in one liter of solution. The pH was adjusted to 3.5 with HCl and measured by means of a Beckman glass electrode.

To analyze for reducing sugars, the method of FOLIN and WU (11) was used. The standard was a solution of galactose, so that the results are expressed in terms of galactose, but it must not be assumed that all or any of the reducing sugar is in that form.

Two methods of preparation of inoculum were employed. When the organism was first obtained, transfers were made to Czapek's agar, using the stab transfer method. These cultures were allowed to grow at room temperature for a few days and then were placed in a refrigerator maintained at approximately 10° C. Prior to trials, one tube was removed from the refrigerator and allowed to stand at room temperature overnight, and inoculations were made directly from this stock culture. In the second method an "activated" culture was used. The activation consisted in first transferring the organism from the stock culture to a flask containing 25 ml. of basal nutrient and 2.5 gm. of arabogalactan. The mold was allowed to grow for 10 days at 28° C. before using it as a source of inoculum.

Inoculations were made from either source to 25 ml. of basal nutrient con-

taining 10 per cent arabogalactan. In all experiments 250-ml. titration flasks were used, stoppered with rolled plugs of nonabsorbent cotton. The flask and contents were sterilized in a steam-pressure cooker at 15 pounds per square inch for 20 minutes, and inoculations were made in a sterilized transfer cabinet.

At the end of each period of investigation the mold cultures were killed by the addition of 10 ml. of a 0.1 per cent mercuric chloride solution saturated with thymol. The mycelium was filtered off, washed with distilled water until the total volume of the filtrate and washings was about 100 ml., and then each solution was brought to volume in 100-ml. volumetric flasks. Aliquot portions of these solutions were used for analyses. If mold weights were desired, the mycelium was filtered on a weighed sintered glass crucible and dried to constant weight at 70° C.

Controls were run in all series. All pH values were checked with a glass electrode.

Results

Effect of pH

To determine the effect of variation in pH, a series of flasks was set up so that the pH varied one-half a unit from pH 2.0 to 5.0. The temperature of these runs was 35° ± 0.5° C. The reducing value was determined at the end of 1, 3, 5, 8, 12, and 16 days. A maximum reducing value of 6.2 mg. of galactose per gram of arabogalactan in the original substrate was obtained at a pH of 3.5 in 5 days. Other maxima were: at pH 2.0, 2.9 in 1 day; at pH 2.5, −8.5 in 16 days; at pH 3.0, −6.2 in 16 days; at pH 4.0, 2.8 in 5 days; at pH 4.5, 5.2 in 3 days; and at pH 5.0, −0.4 in 1 day. The decrease in reducing values at low pH values resulted from the fact that there was some acid hydrolysis of arabogalactan during sterilization, so that the mold grew on the liberated galactose or other degraded sugar residue. When the experimental value was subtracted from the control value, a negative number resulted. The negative value at pH 5.0 merely reflected the poor growth of the mold at that pH and was barely beyond the limits of precision of the method.

Effect of temperature

In this series the pH value was kept constant at 3.5 and the temperature was varied. Flasks were removed from the constant temperature airbath for analysis at the end of 3, 5, 8, and 12 days. A maximum reducing value of 12.6 gm. of galactose per gram of arabogalactan was obtained at 28° C. in 8 days. A value nearly as great, 12.0, was obtained at 30° in the same period of time. Analysis of the growth curves in each case indicates that there is little to choose between these two temperatures. At 25° C. the maximum of 9.4 was obtained in 8 days; at 35° C. the maximum of 6.2 was obtained in 5 days.

Effect of method of inoculum preparation

With the temperature at 28° C. and the pH at 3.5 the maximum reducing value obtained with the stock culture was 12.6 in 8 days, while the maximum reducing value with the activated culture was 7.9 in 8 days. This would indicate that successive transfers from a culture of the mold grown on arabogalactan were not beneficial, differing from Funke's (14) finding with diastase produced by *A. niger*.

Effect of heavy metals

Heavy metals, particularly zinc, manganese, molybdenum, copper, gallium,

and iron, have been reported effective in increasing the rate of growth of *A. niger* (5, 12, 15, 22, 29, 35, 47, 48, 49, 52, 59). When the temperature was kept constant at 28° C. and the pH at 3.5, the data in table 1 were obtained at 4 and 6 days. Judging from the values given in the table, all the heavy metals mentioned (except gallium) are effective in increasing the rate of growth of the tested strain when it is grown on arabogalactan. Except for gallium and molybdenum, no data on the individual heavy metals have been obtained under these conditions.

TABLE 1

EFFECT OF HEAVY METALS ON GROWTH OF A. NIGER GROWN ON ARABOGALACTAN

BASAL NUTRIENT	GROWTH (MG. MYCELIUM)		REDUCING VALUE (MG. GALACTOSE/GM. ARABOGALACTAN)	
	4 DAYS	6 DAYS	4 DAYS	6 DAYS
No supplement.....	4*	6*	2.6*	3.1*
Supplemented with Fe, Cu, Mn, Zn†..	10*	15*	4.0*	8.6*
Supplemented with Fe, Cu, Mn, Zn, Mo†.....	20	58	.....	.....
Supplemented with Fe, Cu, Mn, Zn, Mo, Ga†.....	19	.....	.....	.....

* Interpolated from growth curve.

† Per liter of nutrient: Fe 0.2 mg., Cu 0.04 mg., Mn 0.02 mg., Zn 0.14 mg., Mo 0.02 mg., Ga 0.02 mg.

EFFECT OF YEAST

Yeast has been recognized as a growth stimulant for molds by many investigators, although there seems to be some disagreement about the actual part it plays in causing increase in growth (13, 21, 48, 50, 51). Considering the success that others had experienced, it was thought desirable to include experiments with yeast extract in the nutrient. The amount used was that obtained from 250 mg. of yeast[1] per 25 ml. of nutrient. The extract represented 95 mg. of solid matter, or 38 per cent of the total yeast.

When the pH was kept at 3.5 and the temperature at 28° C., yeast caused the mycelium to reach a weight of 183 mg. in 4 days, compared with only 20 mg. obtained with the control. Similar striking results were obtained when the nutrient contained glucose instead of arabogalactan. With glucose the control mycelium weighed 85 mg. after 44 hours, while the mycelium grown in presence of the yeast extract weighed 508 mg. Yeast evidently contains a factor or factors which stimulate the growth of *A. niger*, and this stimulation is not dependent upon the presence of some esoteric carbohydrate, such as arabogalactan, but is effective even with glucose, which is considered a satisfactory growth material when supplemented by the proper minerals.

Although it was not the purpose of this study to investigate the nature of this stimulant, a few experiments along that line were tried. The results showed that the stimulants were soluble in 70 per cent alcohol, and that they were not adsorbed completely on Lloyd's reagent from acid or alkaline solutions. A partial adsorption was obtained at pH 1.5 and 5.0. The factor(s) were completely adsorbed on charcoal but could not be eluted readily by use of acetone and ammonia, as recommended by ROBBINS (36) for his Z factor. The incomplete adsorption on Lloyd's reagent indicates that the factor is not folic acid (28), which is adsorbed on that material. Whether it is the new factor reported by SNELL and PETERSON (44) is questionable, since STOKSTAD (51) has offered

[1] Northwestern Yeast Company, Chicago, Illinois.

TABLE 2

VARIATIONS IN GROWTH RATE OF A. NIGER ON SUPPLEMENTED MEDIA CONTAINING ARABOGALACTAN. TIME OF INCUBATION 4 DAYS UNLESS OTHERWISE INDICATED. RESULTS ARE AVERAGES OF DUPLICATE CULTURES

SUPPLEMENT TO BASAL NUTRIENT	CONCENTRATION OF SUPPLEMENT (MG./25 ML.)	MYCELIUM WEIGHT MINUS CONTROL (MG.)
1. Thiamin	0.2	8*
2. Thiamin	0.4	— 1*
3. Riboflavin	0.05	— 11
4. Riboflavin	0.2	— 22*
5. Nicotinic acid	0.05	— 11
6. Nicotinic acid	0.1	— 6
7. Nicotinic acid	0.2	— 8*
8. Calcium pantothenate	0.025	34*
9. Calcium pantothenate	0.05	— 3
10. Calcium pantothenate	0.1	— 7
11. Pyridoxine	0.025	— 5
12. Pyridoxine	0.05	— 2
13. Pyridoxine	0.1	43*
14. Ascorbic acid	1.0	— 14*
15. Ascorbic acid	2.0	— 17*
16. Asparagine	125.0	— 15
17. Asparagine	250.0	— 13
18. Pyruvic acid	0.5	— 7
19. Pyruvic acid	1.0	— 3
20. Glycolic acid	0.15	— 6
21. Glycolic acid	0.30	— 7
22. Glutamic acid	0.25	— 19*
23. Glutamic acid	0.50	— 21*
24. Pyruvic acid	0.5	.....
Glycolic acid	0.15	— 14*
25. Thiamin	0.2	.....
Pyruvic acid	0.5	— 3
Pyridoxine	0.1	.....
26. Thiamin	0.2	.....
Calcium pantothenate	0.025	— 3
Pyridoxine	0.1	.....
27. Ascorbic acid	4.0	.....
Pyruvic acid	0.5	15
Glycolic acid	0.15	.....
28. Thiamin	0.2	.....
Riboflavin	0.05	.....
Nicotinic acid	0.1	— 7
Calcium pantothenate	0.025	.....
Pyridoxine	0.05	.....
29. Biotin	0.0001	0
30. Biotin	0.0003	— 3
31. Acid-hydrolyzed casein	10.0	10
32. Acid-hydrolyzed casein	20.0	6
33. Yeast extract	95.0	163

* Six-day growth.

evidence to show that it is a nucleotide, and our incomplete results indicate that the active factor(s) in the present experiments are not nucleotides.

EFFECT OF OTHER GROWTH STIMULANTS

Several experiments were run in an effort to determine whether the growth factors in yeast were known members of the vitamin B complex, mixtures of amino acids, or other growth stimulants. For these experiments the usual nutrient and heavy metal supplement were used at pH 3.5 and temperature of 28° C. The materials to be tested were added at the concentration levels noted in tables 2 and 3 and were chosen on the basis of their known presence in yeast or because they

TABLE 3

INFLUENCE OF YEAST AND CASEIN ON GROWTH OF A. NIGER USING GLUCOSE AS SOURCE OF CARBON. TIME OF INCUBATION 44 HOURS. INOCULATED FROM STOCK. RESULTS ARE AVERAGES OF TRIPLICATE CULTURES

SUPPLEMENT TO BASAL NUTRIENT	QUANTITY ADDED (MG./25 ML.)	MYCELIUM WEIGHT MINUS CONTROL (MG.)
1. Casein	10	.....
Cystine	5	19
2. Casein	10	.....
Cystine	5	23
Vitamins*	0*	.....
3. Yeast extract	9.5	135
4. Casein	100	.....
Cystine	5	203
5. Casein	100	.....
Cystine	5	161
Vitamins*	0*	.....
6. Casein	80	.....
Cystine	5	234
Yeast nucleic acid	10	.....
Inositol	10	.....
7. Yeast extract	95	423
8. p-aminobenzoic acid	0.00025	2
9. p-aminobenzoic acid	0.0025	7
10. p-aminobenzoic acid	0.025	3

* Vitamins added per 25 ml. were: thiamin 200 μ gm.; calcium pantothenate 25 μ gm.; pyridoxine 100 μ gm.

had been used successfully by others (1, 2, 6, 9, 18, 23, 24, 25, 30, 31, 32, 37, 38, 39, 40, 41, 42, 43, 45, 46, 50, 54). None of the known water-soluble vitamins, including biotin, p-aminobenzoic acid, and inositol, caused significant increase in the growth rate of the mold. The amino-acid mixture obtained from the acid hydrolysis of casein did increase the growth rate, but the increase was well below that noted with yeast extract. This gives ample evidence that yeast supplies some agent not yet known as a growth factor for *A. niger*.

Effect of source of arabogalactan

All this work was done using arabogalactan obtained either from an old larch stump or from the butt log of a larch tree 25 cm. in diameter. The viscosity-concentration coefficient of the arabogalactan from either source was the same, 5.8×10^{-3}, and the biological activity as measured by growth of *A. niger* seemed the same. A third sample of arabogalactan taken from a tree about 95 cm. in diameter had a viscosity-concentration coefficient of 5.1×10^{-3}. It permitted somewhat more rapid growth of the mold. Controlled experiments using the last of the older samples of arabogalactan gave mycelial weights of 187 mg. in 6 days, while the less viscous arabogalactan permitted the mold to grow to 273 mg. A fourth sample of arabogalactan collected from another large tree permitted growth of 270 mg. of mycelium in the same experiment. The last two samples showed the greatest difference in viscosity effect, yet have about the same effect on the growth rate of the mold. The earlier samples, with an intermediate viscosity effect, do not support nearly such vigorous growth.

The reason for this is not yet apparent. It is not related to viscosity characteristics or to season, because the third sample was collected from a tree cut in June, 1941, and the fourth sample was obtained from a tree cut in October, 1941. The data are still too few to justify a fair conclusion, however.

Effect of metabolites

The mold during growth on arabogalactan will produce some metabolites different from those developed when the mold is grown on sucrose. One of these, galactose, is believed to be a poor source of carbon for molds (8, 19, 55). From similar experiments Waterman (54) ranks galactose and galactans with poisons such as copper sulphate, and narcotics like salicylic acid, in causing decreased growth, loss of color, and decreased sporulation.

One series of experiments involved the comparison of mycelial development on the ordinary nutrient with 10 per cent arabogalactan, heavy metals, and yeast for the controls and a similar medium containing 0.2 per cent galactose in addition. This concentration of galactose was arrived at by assuming that the reducing values found were due to galactose. Although this assumption is false, it gives the maximum possible concentration of that sugar likely to be found in the media. The mycelial weights averaged 428 mg. in the first case and 500 mg. in the second, showing that the toxic effect of galactose was not a problem in these experiments.

In the absence of yeast, however, it has been found that a second mycelial mat generally does not develop on the same nutrient after one mat has been removed, indicating that some toxic metabolic product is formed by the mold. No information is available on the nature of this product.

In the presence of the yeast extract, however, a second mat can be grown in about the same time as required for the first one. Evidently one of the functions of the yeast extract is either to combine with the toxic material in some way or to prevent its formation.

Discussion

Whether *A. niger* develops a specific galactanase when grown on arabogalactan has not been determined, but the optimum conditions for the growth of the mold have been established, and work is now possible on the concentration of the enzyme which hydrolyzes the galactan.

Except for the differences in the weights of the mycelia, the results obtained with *A. niger* grown on arabogalactan are very similar to those obtained by STEINBERG (48–50), using glucose or sucrose in the substrate. He has reported gallium as a necessary element, which disagrees with the results reported here; but no attempt was made to exclude gallium, and it might have been present in small quantities in some of the c.p. salts used in the nutrient.

Yeast extract contains some agent or agents which stimulate the growth of *A. niger* in much the same way that riboflavin stimulates the growth of *Lactobacillus casei*. There is some evidence that yeast either prevents the formation of or combines with toxic metabolic products of the mold grown on arabogalactan.

It has been shown that the water-soluble material in yeast differs from any of the known vitamins of the B complex or from growth stimulants ordinarily associated with molds. Evidently the strain of *A. niger* used in this work is capable of synthesizing many of the vitamins recommended for other strains. It cannot be considered that this stimulating effect of yeast is due to amino acids or nucleic acid, inasmuch as a casein hydrolysate or nucleic acid with or without vitamins was much less effective than yeast extract at the same concentration of total solids.

Little can be said at this time regarding the poor growth of the mold on arabogalactan. Since the molecule is either spherical or slightly ellipsoidal, the galactosidic linkages may be folded into the structure in such a way that they cannot readily make contact with the enzyme, which would prevent catalysis. On the other hand, the galactosidic linkages themselves may offer the steric hindrance preventing proper contact between the substrate and enzyme, in the same way that the branching of glucosidic chains in starch hinders the activity of certain amylases. The slow rate of hydrolysis and the biological variability of arabogalactan warrant further investigation.

Summary

1. The optimum conditions for growth of *Aspergillus niger* (Thom 3528.7) on a substrate containing arabogalactan, CURRIE'S salt medium supplemented with salts of iron, zinc, copper, manganese, and molybdenum, and a yeast extract are a pH of 3.5 and a temperature between 28° and 30° C.

2. *Aspergillus niger* produces an enzyme capable of hydrolyzing arabogalactan; whether or not this is a specific galactanase has not been determined.

3. There is a growth factor or factors not yet identified in yeast.

We express our thanks to Merck and Company for the vitamins used in this work, to Dr. PAUL GYÖRGY, Western Reserve University, for a generous

sample of biotin concentrate, and to Dr. JOHN EHRLICH, University of Idaho, for mold cultures and advice during the course of this investigation. This work was done at the Department of Chemistry, University of Idaho.

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LITERATURE CITED

1. AKASI, SYUSE, Preparation of pure nucleic acid from molds and its characterization. Jour. Biochem. (Japan) 29:21–29. 1939.
2. BERNHAUER, K., GÖRLICH, B., and KOCHER, E., Formation of vitamin C-like substances by molds and bacteria. Biochem. Zeitschr. 286: 60–65. 1936.
3. BIERRY, H., and GIAJA, J., Enzymes which attack mannans, galactans, and celluloses. Biochem. Zeitschr. 40:370–389. 1912.
4. ———, and ———, The digestion of mannans and galactans. Compt. rend. Acad. Sci. (Paris) 148:507–510. 1909.
5. BORTELS, HERMAN, The significance of iron, zinc, and copper for microorganisms. Biochem. Zeitschr. 182:301–358. 1927.
6. BRÜNNING, EMIL, Wachstum und Stickstoffassimilation bei *Aspergillus niger* unter dem Einfluss von Wachstums-regulatoren und von vitamin B. Ber. deutsch. Bot. Ges. 52:423. 1934.
7. CURRIE, J. N., The citric acid fermentation of *Aspergillus niger*. Jour. Biol. Chem. 31:15–37. 1917.
8. EDGECOMBE, A. E., The effect of galactose on the growth of certain fungi. Mycologia 30:601–624. 1938.
9. FAWNS, H. T., and JUNG, ALBERT, The mineral content of the skin of rats suffering from beriberi, with investigations of the formation of vitamin B_1 by molds. Biochem. Jour. 27:918–933. 1933.
10. FLORY, P. J., and STICKNEY, P. B., Viscosities of polyester solutions and the Staudinger equation. Jour. Amer. Chem. Soc. 62:3032–3038. 1940.
11. FOLIN, O., Two revised copper methods for blood sugar determination. Jour. Biol. Chem. 82:83–93. 1929.
12. FOSTER, J. W., The heavy-metal nutrition of fungi. Bot. Rev. 5:207. 1939.
13. FRIES, NILS, Über die bedeutung von Wuchsstoffen für das Wachstum verschiedener Pilze. Symb. Bot. Upsal. 3:1–188. 1938.
14. FUNKE, G. L., Researches on the formation of diastase by *Aspergillus niger* van Tieghem. Rec. Trav. Botan. Néerl. 19:219–275. 1922. (Rev. App. Mycol. 2:330. 1923.)
15. GOLLMICK, FRIEDRICH, The influence of zinc, copper, and iron, and their combination on the growth of *Aspergillus niger*. Zentr. Bakt. Parasitenk. II Abt. 93:421–442. 1936.
16. GORTNER, R. A., Outlines of biochemistry New York. 1938.
17. HERRISEY, H., Recherches chimiques et physiologiques sur la digestion des mannanes et des galactanes par la seminase chez lez végétaux. These, Paris. 1903.
18. HIDA, T., Production of dimethylpyruvic and pyruvic acids by *Aspergillus niger*. Jour. Shanghai Sci. Inst. 1:201–214. 1935. (C.A. 30: 5606. 1936.)
19. HORR, W. H., Utilization of galactose by *Aspergillus niger* and *Penicillium glaucum*. Plant Physiol. 11:81–89. 1936.
20. HOSSFELD, R. L., The action of several strains of *Aspergillus niger* on the aqueous extract of western larch heartwood. Master's Dissertation, University of Idaho. 1939.
21. HUEBER, E., Zur physiologie einiger arten von *Aspergillus*. Beih. Bot. Centralbl. Abt. 58:173. 1938.
22. KAUFFMANN-COSLA, O., and BRÜLL, R., The biological importance of the ions of zinc, iron, and potassium in growth and metabolism. Arch. Intern. Pharmacodynamie 57:188–194. 1937.
23. KOSER, S. A., and SAUNDERS, FELIX, Accessory growth factors for bacteria and related microorganisms. Bact. Rev. 2:99–160. 1938.
24. LAVOLLAY, J., and LABOREY, F., Effect of ascorbic acid on the pigmentation of the mycelium of *Aspergillus niger* and on the development of this fungus. Compt. rend. Acad. Sci. (Paris) 205:179. 1937.
25. LEONIAN, L. H., and LILLY, V. G., Studies on the nutrition of fungi. I. Thiamin, its constituents, and the source of nitrogen. Phytopath. 28:531–548. 1938.
26. LUTZ, L., The enzymes of the fungus Hymenomycetes: the hydrolysis of hemicelluloses. Compt. rend. Acad. Sci. (Paris) 190:892–895. 1930.
27. ———, Hydrolytic enzymes secreted by the fungi Hymenomycetes. Degradation of the cell membrane. Bull. Soc. Chem. Biol. 13:436–457. 1931.
28. MITCHELL, H. K., SNELL, E. E., and WILLIAMS, R. J., The concentration of folic acid. Jour. Amer. Chem. Soc. 63:2284. 1941.
29. NIELSEN, NIELS, and HARTELIUS, VAGN, The co-growth effect of certain metal mixtures. Biochem. Zeitschr. 276:183–185. 1935.
30. ———, and ———, Chemistry of growth substance B. Nature (London) 138:203. 1936.

31. ———, and ———, Separation of growth-promoting factors for yeast and molds. Compt. rend. Trav. Lab. Carlsberg, Ser. Physiol. 22: (1): 1937. 22 pp. (C.A. 31:6280. 1937.)
32. ———, and ———, The influence of β-alanine on the growth of *Aspergillus niger*. Biochem. Zeitschr. 296:171–173. 1938.
33. NORMAN, A. G., The biological decomposition of plant materials. IX. The aerobic decomposition of hemicelluloses. Ann. Appl. Biol. 21:454–475. 1934.
34. OWENS, H. S., The viscosities of arabogalactan solutions. Jour. Amer. Chem. Soc. 62:930–932. 1940.
35. PIRSCHLE, KARL, Comparative investigations on the physiological effect of the elements as shown by growth experiments with *Aspergillus niger*. Planta 23:177–224. 1934.
36. ROBBINS, W. J., Further observations on factor Z. BOT. GAZ. 102:520–535. 1941.
37. ———, Pyridine analog of thiamine and the growth of fungi. Proc. Nat. Acad. Sci. 27:419–422. 1941.
38. ROBBINS, W. J., and KAVANAGH, F., Vitamin B_1, or its intermediates, and the growth of certain fungi. Amer. Jour. Bot. 25:229. 1938.
39. ———, and ———, Thiazole effect on *Phycomyces*. Proc. Nat. Acad. Sci. 27:423–427. 1941.
40. SAKURAI, KYUYA, Vitamin B_1 synthesis by microorganisms. I. Molds. Jour. Sci. Hirosima Univ. Ser. B. 3:191–200. 1939. (C.A. 33:6901. 1939.)
41. SCHELLING, NELLEY J., Growth stimulation of *Aspergillus niger* by a vitamin B preparation. Bull. Torr. Bot. Club 52:291–310. 1925.
42. SCHOPFER, W. H., and JUNG, A., Growth factors and vitamins in plants. Effect of extracts of *Aspergillus* on the development of *Phycomyces*. Arch. Microbiol. 6:334–344. 1938.
43. SKINNER, C. E., The synthesis of aromatic amino acids from inorganic nitrogen by molds and the value of mold proteins in diets. Jour. Bact. 28:95–106. 1934.
44. SNELL, E. E., and PETERSON, W. H., Growth factors for bacteria. Jour. Bact. 39:273–285. 1940.
45. SOLHEIM, W. G., SEARS, S. S., and COLLINS, R. C., Effect of vitamins on the growth of fungi in pure culture. Phytopath. 23:929. 1933.
46. STEINBERG, R. A., Relation of accessory growth substances to the heavy metals, including molybdenum, in the nutrition of *Aspergillus niger*. Jour. Agr. Res. 52:439. 1936.
47. ———, The essentiality of gallium to growth and reproduction of *Aspergillus niger*. Jour. Agr. Res. 57:569–574. 1938.
48. ———, Relation of carbon nutrition to trace-element and accessory requirements of *Aspergillus niger*. Jour. Agr. Res. 59:749–763. 1939.
49. ———, Growth of fungi in synthetic nutrient solutions. Bot. Rev. 5:327–350. 1939.
50. ———, Relation of accessory substance and amino requirements to the carbon nutrition of *Aspergillus niger*. Rept. Proc. 3d Internat. Congr. Microbiol. 491. 1940.
51. STOKSTAD, E. L. R., Isolation of a nucleotide essential for the growth of *Lactobacillus casei*. Jour. Biol. Chem. 139:475–476. 1941.
52. VANGHELOVICI, M., and SERBAN, F., Biosynthesis of sterols by *Aspergillus niger*. Bull. Sect. Sci. Acad. Roumaini 22:287–292. 1939–1940. (C.A. 34:6966. 1940.)
53. WAKSMAN, S. A., and DIEHM, R. A., On the decomposition of hemicelluloses by microorganisms. II. Decomposition of hemicelluloses by fungi and actinomyces. Soil Sci. 32:97–117. 1931.
54. WATERMAN, H. J., Mutation bei *Penicillium glaucum* und *Aspergillus niger*. Zeitschr. Gärungsphysiologie 3:1–15. 1913.
55. WENT, F., Course of formation of diastase by *Aspergillus niger*. Proc. Acad. Sci. Amsterdam 21:479–493. 1919.
56. WHITE, E. V., Constitution of arabo-galactan. I. Jour. Amer. Chem. Soc. 63:2871–2875. 1941.
57. ———, Constitution of arabo-galactan. II. Jour. Amer. Chem. Soc. 64:302–306. 1942.
58. ———, Constitution of arabo-galactan. III. Jour. Amer. Chem. Soc. 64:1507–1511. 1942.
59. WOLFF, L. K., and EMMERIE, A., Growth of *Aspergillus niger* and the copper content of the nutritive medium. Nederland Tydschr. Hyg. Microbiol. Serol. 5:66–75. 1930. (Biochem, Zeitschr. 228:443–450. 1930.)

TREATMENT OF SEEDS WITH SYNTHETIC GROWTH-REGULATING SUBSTANCES

WILLIAM S. STEWART AND CHARLES L. HAMNER[1]

(WITH TWO FIGURES)

Introduction

It has been suggested that increased growth of plants may be obtained by treating the seeds with growth regulators. Results of some experiments seem to support this contention (1, 2, 3, 5, 8, 10, 11, 13, 14, 17, 20), but other investigations have failed to give corroboration of such results (4, 6, 7, 9, 12, 15, 16, 18, 19). This confusion may be due in part to differences in the environmental factors under which the various investigations were made. Until enough data are available from varied localities, it is not possible to estimate conclusively the merits of treating seeds with growth-regulating substances, and much additional evidence is still required.

The experiments reported here concern the effects of treatment of seeds with a number of synthetic growth-regulating substances and combinations of them over a wide range of concentrations.

Methods

These experiments were conducted at the U.S. Bureau of Plant Industry Station, Beltsville, Maryland; at the University of Chicago; and at Lake Geneva, Wisconsin. Growth-regulating substances were applied either by dusting the seeds with the substance dispersed in talc dust or by soaking them in water solutions of the substances.

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GREENHOUSE EXPERIMENTS

Greenhouse experiments were conducted at Chicago and at Beltsville. To permit a survey of the effects of various growth-regulating substances, attention was confined at Chicago to oat plants and at Beltsville to radish. These experiments were carried out from January to June, 1942. The experimental design, treatments, and materials used were similar at the two localities.

Preparations of the substances in talc were made by dissolving the substance in 50 cc. of 95 per cent ethyl alcohol and adding this to 50 gm. of powdered talc, forming a thin paste. The alcohol was then evaporated off in a drying oven at 80° C. and the dried cake finely pulverized. Water solutions of the substances were prepared by dissolving them directly in water. Those that did not dissolve easily were first dissolved in a few cubic centimeters of ethyl alcohol and then added to the water. Except where noted, the seeds were soaked for 14 hours in these solutions.

A randomized block statistical design was used. With radish each block was replicated seven times over benches which extended through the middle of the greenhouse. The experiments with oat were usually replicated ten times. Across each end of each bench was a "guard" row of plants grown from untreated seeds. Each block consisted of nine treatments of six pots each across the bench. There were four seeds per pot

in the case of radish and five seeds in the case of the oat, making a total of 1512 seeds in each experiment with radish and 2700 seeds in each experiment with oat.

Six-inch clay pots filled with a potting mixture containing five parts loam, two parts sand, and one part manure, were used with radish. The pH of the mixture was 7.0. After planting, the seeds were firmed in and covered with a light top dressing of one part sand and one part loam. The oat grains were planted in a similar potting mixture in 1½-liter glazed crocks. A ¼-inch top dressing of pure quartz sand was used. Both the oat and radish were watered whenever required. For radish the greenhouse temperature was maintained as nearly as possible at 60°–65° F. for the night and at 70°–75° F. in the daytime. For oat it was 55°–60° F. at night and 70°–75° F. in the daytime.

In one greenhouse experiment radish seeds were treated with commercial preparations of growth-regulating substances and were grown in three types of soil: a sandy loam (pH 5.5), a potting mixture (pH 7.0), and a muck mixture (pH 5.2). Untreated radish seeds were also grown in these different soil mixtures but were watered every 4 days with vitamin B_1 at a concentration of 0.01 mg. per liter in one series and 8.00 mg. per liter in another.

The radish plants were usually grown for 28 days before harvesting. In three experiments this was exceeded by a day or so, while in a few others the plants were harvested before this time, but all the plants in any one experiment were harvested on the same day. Germination counts were made 1 week after planting.

The oat plants were grown about 42 days before harvesting. Germination counts were made 14 days after planting.

At harvest, the fresh weight of the tops and roots of radish was measured to the nearest ±0.5 gm., the roots having been previously washed free from soil. The tops were then placed directly into a forced-draft electric drying oven at 80° C. The roots were sliced in thin sections, spread out on paper, and similarly dried. After both the tops and roots were brittle dry, about 24 hours, they were taken from the oven and allowed to cool to room temperature. They were then weighed to within ±0.05 gm. on a torsion balance.

The oat tops were cut from the roots at ground level. Height and fresh weight measurements were taken. The tops were then dried and weighed similarly.

Field experiments

All the field experiments were performed at Beltsville, from April to September, 1942, except for duplicate plantings of radish and sugar beet at Lake Geneva, Wisconsin.

The soil used at Beltsville is a light sandy clay loam, with pH 5.3. The soil has been in cultivation for several years and prior to the spring plowing received one application of rotted manure. At planting time the 6-acre area was fertilized with a 5–8–5 artificial fertilizer at about 300 lb. per acre. The soil was prepared and crops planted according to usual farm practice.

Buckwheat, corn, oat (var. Fulghum), and Zuchini squash were planted in Latin squares for statistical analysis. The other crops were planted in rows as randomized blocks, with a minimum of eight replications. During a spring drought irrigation was necessary for 3 weeks.

The radish and sugar beet seeds grown at Lake Geneva were treated with the same growth-substance preparations used at Beltsville and were grown in rich

loam soil which had a pH of 7.3. They were planted in rows as randomized blocks, each replicated six times. The cross checked. After germination each hill was thinned to three plants. The field was cross cultivated.

TABLE 1

AVERAGE DRY WEIGHT IN GRAMS OF RADISH PLANTS FROM SEEDS DUSTED WITH GROWTH-REGULATING SUBSTANCES IN TALC. GERMINATION AT LEAST 90 % IN ALL CASES. EACH FIGURE AVERAGES ABOUT 150 PLANTS

Exp. no.	Growth-regulating substance	Seeds not dusted	Seeds dusted (as p.p.m. in talc)						
			0	2	20	200	1000	10,000	30,000
1.	Indolebutyric acid	0.729	0.832	0.683	0.693	0.714	.....	.....	.....
9.	Indolebutyric acid	.657	.741	.....	.....	.....	0.769	0.738	0.741
2.	Naphthalene acetamide	.773	.776	.753	.770	.794	.....	.....	.....
9.	Naphthalene acetamide	.657	.741	.....	.....	.....	.831	.734	.729
3.	Napthoxyacetic acid	.574	.577	.564	.561	.574	.....	.....	.....
10.	Napthoxyacetic acid	.614	.638	.....	.....	.....	.631	.702	.636
4.	Phenylacetic acid	.763	.752	.791	.749	.757	.....	.....	.....
6.	Phenylacetic acid	.613	.600	.....	.....	.....	.585	.578	.590
5.	Naphthaleneacetic acid	.854	.885	0.892	0.905	0.848	.....	.....	.....
6.	Naphthaleneacetic acid	0.613	0.600	.....	.....	.....	0.555	0.458	0.460

TABLE 2

AVERAGE DRY WEIGHT IN GRAMS* OF RADISH PLANTS FROM SEEDS SOAKED 14 HOURS IN WATER SOLUTIONS OF GROWTH-REGULATING SUBSTANCES

Exp. no.	Growth-regulating substances	Seeds not soaked		Seeds soaked (as p.p.m.)							
				0		10		100		200	
		Weight (gm.)	Germination (%)	Weight (gm.)	Germination (%)	Weight (gm.)	Germination (%)	Weight (gm.)	Germination (%)	Weight (gm.)	Germination (%)
1.	Indolebutyric acid	0.729	96.4	0.726	97.6	0.723	90.5	0.598	88.1	.....	.....
2.	Naphthalene acetamide	.773	97.0	.832	92.2	.786	89.3	.581	96.4	.....	.....
3.	Naphthoxyacetic acid	.574	92.8	.570	91.7	.533	95.2	.278	80.9	.....	.....
4.	Phenylacetic acid	.763	97.0	.765	94.0	.749	91.7	.750	86.3	0.724	90.5
5.	Naphthaleneacetic acid	0.854	92.3	0.922	91.1	0.793	87.5	0.424	81.5	0.160	47.6

* Average dry weight = $\frac{\text{Total dry weight}}{\text{Germination percentage} \times 168}$.

yields of all the farm crops except corn and sugar beet were weighed to within ± 50 gm.

The forty-nine individual corn plots within a square, each consisted of twenty hills. The hills were 3 feet apart and were

Two of the four Latin squares of corn received the usual application of manure as well as an application of about 400 lb. per acre of 5–8–5 fertilizer after plowing. At planting, another application of the fertilizer was made at each hill. The oth-

er two squares received no manure or fertilizer.

The fresh weight of the top growth of the corn was determined to within ±250 gm. on a large platform balance. The dry weight was determined from a quart sample of chopped material (fodder) from five plants out of each plot. It was dried at 80° C. for 96 hours in a forced-draft electric drying oven.

TABLE 3

GROWTH OF RADISH PLANTS WATERED WITH VITAMIN B_1 OR GROWN FROM SEEDS DUSTED WITH COMMERCIAL PREPARATIONS OF GROWTH-REGULATING SUBSTANCES

Exp. no.	Seed treatment	Sandy soil loam		Potting soil mixture		Muck soil mixture	
		Germination (%)	Average dry weight* (gm.)	Germination (%)	Average dry weight (gm.)	Germination (%)	Average dry weight (gm.)
7.	a. Untreated	90.4	0.370	87.5	0.720	96.4	1.116
	b. Commercial preparation A	90.5	.437	95.2	.736	93.4	1.090
	c. Commercial preparation B	92.9	.374	92.9	.645	94.6	1.127
	d. Watered seven times with 0.01 mg./l. vitamin B_1	80.4	.364	91.1	.784	94.0	1.064
	e. Watered seven times with 8.00 mg./l. vitamin B_1	86.3	0.353	90.5	.670	92.3	1.086
9.	a. Untreated	.....	.....	91.2	.657	.....	.....
	b. Commercial preparation C	.....	.....	92.8	0.749	.....	.....

* Average dry weight = $\frac{\text{Total dry weight}}{\text{Germination percentage} \times 168}$.

Results

GREENHOUSE EXPERIMENTS

With radish there were no statistically significant increases in either fresh or dry weight of the tops or roots of plants from any of the treated seeds over those from untreated (tables 1–4). When the seeds were soaked 14 hours in a water solution containing 500 p.p.m. indolebutyric acid, strong inhibition of growth was noted (fig. 1). Inhibition of growth is indicated with naphthaleneacetic acid at 10 p.p.m. (table 2).

The only response of radish that might be interpreted as increased growth was from soaking the seeds 14 hours in a 100 p.p.m. solution of indolebutyric acid. After 9 days the hypocotyls of the plants from treated seeds were 130 per cent longer than those of plants from untreated seeds (fig. 2), a statistically significant difference at the 1 per cent level. At harvest time, however, the dry weight of the plants with the longer hypocotyls was actually less than that from untreated seeds. Likewise at harvest time the hypocotyls of the plants from the treated seeds were not significantly longer than those from the untreated. This was probably owing to the fact that the plants with the longer hypocotyls were procumbent on the top of the soil and so did not grow as vigorously as did the more erect plants or develop the bulbous swelling characteristic of radish.

As with radish, the treatment of oat grains with growth regulators did not increase their subsequent growth over plants from untreated seeds (tables 5–7).

TABLE 4

GROWTH OF RADISH PLANTS FROM SEEDS TREATED WITH LEVULINIC ACID

EXP. NO.	SEED TREATMENT	GERMINATION (%)	AVERAGE DRY WEIGHT* (GM.)
8.	a. Untreated	95.8	0.547
	b. Dusted with talc dust only	92.8	.550
	c. Dusted with talc dust 1000 p.p.m. levulinic acid	94.0	.558
	d. Same as c, 10,000 p.p.m.	93.4	.579
	e. Same as c, 100,000 p.p.m.	93.4	.527
	f. Soaked 4 hours in water only	92.8	.611
	g. Soaked 4 hours in 1000 p.p.m.†	83.9	.607
10.	a. Untreated	93.6	.614
	b. Soaked 4 hours in water only	92.1	.670
	c. Soaked 4 hours in 500 p.p.m. levulinic acid in water	87.7	.767
	d. Same as c, but leaves sprayed at six intervals with 0.1% levulinic acid in water	90.1	.710
	e. Soaked 4 hours in 1000 p.p.m. levulinic acid in water, and leaves sprayed as in d	82.9	0.710

* Average dry weight = $\frac{\text{Total dry weight}}{\text{Germination percentage} \times 168}$.

† Soaking 4 hours in 10,000 p.p.m. levulinic acid resulted in no germination.

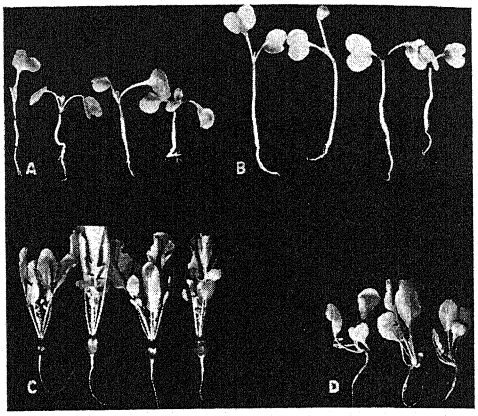


FIG. 1.—Radish plants from seeds soaked in indolebutyric-acid solutions: *A*, plants 11 days old; seeds soaked in water for 14 hours. *B*, same but soaked in 100 p.p.m. indolebutyric-acid solution. *C*, plants 28 days old; seeds soaked in water for 14 hours. *D*, same as *C* but soaked in 500 p.p.m. indolebutyric-acid solution.

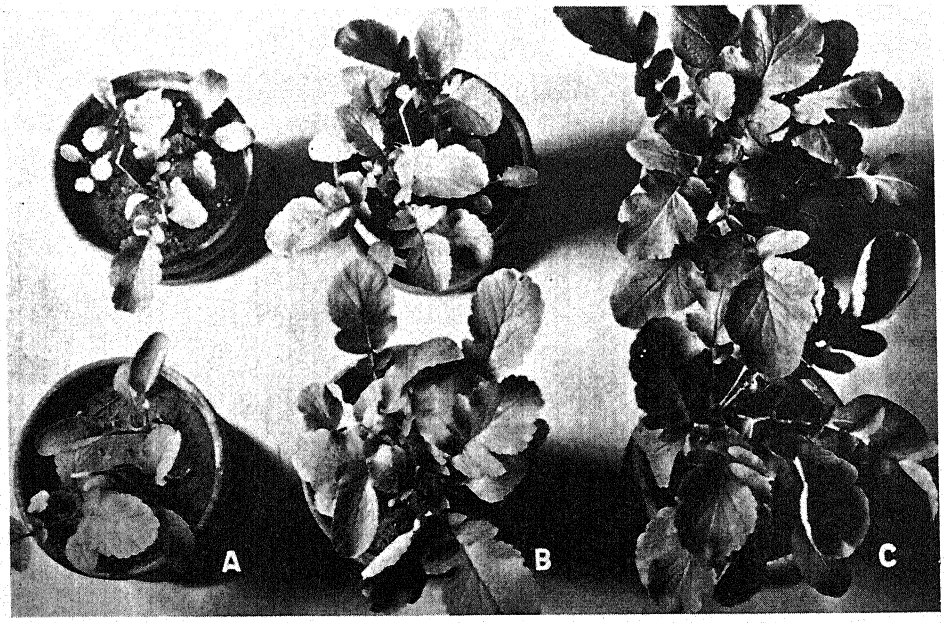


FIG. 2.—Radish plants from untreated seeds after 28 days, showing effect of soil on growth. Grown on: *A*, sandy clay loam soil; *B*, potting soil mixture; *C*, muck soil mixture.

Table 5 shows that although there was no difference due to the treatment, the time of year was very influential in the total growth of the tops. Plants grown in February averaged 0.064 gm. per plant and were about 25 cm. tall after 42 days, while those grown in May and June weighed 0.234 gm. and averaged 35 cm. Soaking seeds in solutions of naphthaleneacetic acid stronger than 500 p.p.m. resulted in decided decrease in germination.

Soaking oat grains in a 3 per cent solution of levulinic acid in water overnight as compared with soaking in water alone decreased germination from 86.6 to 10 per cent. Application of levulinic acid as a spray in conjunction with treatment was ineffective in increasing growth (table 7).

Commercial preparations for seed treatment applied as dust according to manufacturers' directions resulted in no significant increase in growth with either radish or oat (tables 3, 7).

TABLE 5

GROWTH OF OAT PLANTS, AS DRY WEIGHT OF TOPS IN GRAMS, FROM SEEDS DUSTED WITH GROWTH-REGULATING SUBSTANCES. EACH FIGURE AVERAGES ABOUT 260 PLANTS. GERMINATION 80–90% IN ALL TREATMENTS

Exp. no.	Growth-regulating substance	Period of growth	Seeds not dusted	Seeds dusted (as p.p.m. in talc)						
				0	2	20	200	1000	10,000	33,000
1.	Naphthaleneacetic acid	Feb.–Mar.	0.064	0.060	0.060	0.065	0.062	.....	.....	.....
6.	Naphthaleneacetic acid	Apr.–May	.141	.140	.....	.....	.....	0.147	0.134	0.124
3.	Phenylacetic acid	Feb.–Mar.	.107	.109	.106	.107	.104	.....	.....	.....
7.	Phenylacetic acid	Apr.–May	.140	.135	.....	.....	.138	.142	.142	.135
4.	Indoleacetic acid	Mar.–Apr.	.119	.111	0.116	0.111	0.119	.....	.....	.....
6.	Indoleacetic acid	Apr.–May	.141	.140	.....	.....	.....	.134	.140	.141
8.	Indolebutyric acid	Apr.–May	.162	.167	.....	.....	.....	.163	.170	.156
10.	Naphthalene acetamide	May–June	0.234	0.217	.....	.....	.....	0.228	0.185	0.174

TABLE 6

GROWTH OF OAT PLANTS, AS DRY WEIGHT OF TOPS IN GRAMS, FROM SEEDS SOAKED 14 HOURS IN WATER SOLUTIONS OF GROWTH-REGULATING SUBSTANCES. EACH FIGURE AVERAGES ABOUT 270 PLANTS. GERMINATION 85–90% IN ALL TREATMENTS

Exp. no.	Growth-regulating substance	Period of growth	Seeds not soaked	Seeds soaked (as p.p.m.)			
				0	10	100	500
1.	Naphthaleneacetic acid	Feb.–Mar.	0.0642	0.0658	0.0627	0.0641	0.0513
3.	Phenylacetic acid	March	.1076	.1062	.1066	.1084	.1040
4.	Indoleacetic acid	Mar.–Apr.	.1192	.1178	.1179	.1186	0.1234
5.	Indolebutyric acid	Apr.–May	.1620	.1780	.1720	.1680	.....
6.	Naphthalene acetamide	Apr.–May	0.2340	0.2040	0.2260	0.2170	.....

FIELD EXPERIMENTS

Nine different treatments of seeds with growth substance, including various commercial preparations, when tested with nine varied crops failed to produce any increased growth (table 8). These

treatments of the seeds included soaking in water solutions of growth substances, dusting with the substances in powdered talc, and application of commercial preparations of the substances.

Levulinic acid, recently suggested as a growth-regulating substance (10), was applied in a variety of ways to both seeds and plants (tables 4, 7, 9). In no instance did a statistically significant increase in growth or yield occur.

TABLE 7

TOP GROWTH OF OAT PLANTS FROM SEEDS TREATED IN GREENHOUSE WITH PREPARATIONS OF COMMERCIAL GROWTH-REGULATING SUBSTANCES (EXPERIMENT 2, FEBRUARY–MARCH) OR WITH LEVULINIC ACID (EXPERIMENT 9, APRIL–MAY)

EXP. NO.	SEED TREATMENT	GERMINATION (%)	AVERAGE DRY WEIGHT OF TOPS* (GM.)
1.	Untreated	88.8	0.079
2.	Commercial preparation A	89.3	.091
3.	Commercial preparation B	87.0	.080
4.	Commercial preparation C	91.0	.078
5.	Commercial preparation D	87.0	.082
6.	Commercial preparation E	88.8	.082
1.	Untreated	83.8	.166
2.	Soaked 12 hours in water only	86.6	.174
3.	Soaked 12 hours in 0.05% solution of levulinic acid	84.4	.164
4.	Same as 3, but 1% solution	73.8	.177
5.	Same as 3, but 3% solution	10.0	.180
6.	Same as 2, but sprayed weekly with 1 p.p.m. levulinic acid in water solution	85.0	.171
7.	Same as 3, but sprayed weekly with 1 p.p.m. levulinic acid	85.0	.168
8.	Same as 4, but sprayed weekly with 1 p.p.m. levulinic acid	78.3	.179
9.	Same as 5, but sprayed weekly with 1 p.p.m. levulinic acid	7.7	0.163

* Average dry weight = $\frac{\text{Total dry weight}}{\text{Germination percentage} \times 168}$.

U.S. Hybrid 113 and Yellow Dent corn were grown on both fertilized and unfertilized plots. Although the experiment was not designed to compare growth differences between the two, it was observed that the number of full ears of the hybrid was nearly twice the number of the dent corn. The yield of the fertilized corn was greater than that of the unfertilized. There were no significant increases as shown by analysis of variances in fresh or dry weights of stalks, number of ears, or amount of grain as a result of any of the seed treatments (table 10). Again it must be repeated that —as with the other crops—the seed treatments of the two corn varieties resulted in no statistically significant increases in growth or yield, either in the fertilized or the unfertilized crops.

Summary

1. Greenhouse experiments with radish at the U.S. Bureau of Plant Industry Station, Beltsville, Maryland, and with oat at the University of Chicago failed to indicate statistically significant increased growth of plants from seeds treated with growth-regulating substances over those not treated. Indoleacetic acid, indolebutyric acid, phenylacetic acid, naphthaleneacetic acid, and naphthalene acetamide were applied to seeds in talc dust at concentrations from 2 to 33,000 p.p.m. They were also applied by soaking the seeds 14 hours in water solutions of these substances at concentrations from 10 to 500 p.p.m. Application of levulinic acid in a variety of ways to seeds and plants failed to increase their growth over untreated ones. Commercial preparations of growth substances for seed treatment, used according to manufacturers' directions, were similarly ineffective. With radish these chemicals and mixtures were applied to seeds grown on three soil types but caused no significant increased growth of the plants on any of the soils.

2. Field experiments at Beltsville with radish, oat, turnip, sugar beet, soybean, squash, carrot, buckwheat, and corn likewise failed to indicate statistically significant increased growth of plants from seeds treated with seven growth substances, including several commercial

TABLE 8

FIELD CROP TESTS OF SEED TREATMENTS WITH GROWTH-REGULATING SUBSTANCES
GROWTH GIVEN AS AVERAGE FRESH WEIGHT OF ENTIRE PLANT

Exp. no.	Seed treatment	Carrot (gm.)	Radish (gm.)	Sugar beet (gm.)	Turnip (gm.)	Sugar beet* (Wisconsin) (kg.)	Radish (Wisconsin) (gm.)	Squash* (kg.)	Soybean* (kg.)	Buckwheat* (kg.)	Oat* (tops only) (kg.)
1.	Untreated	126.1	8.21	957.0	232.2	19.9	32.3	4.58	19.68	19.59	4.61
2.	Talc dust only	130.1	9.43	.....	.....	.....	.....	.....	.....	.....	.....
3.	Commercial dust preparation A	126.1	8.34	852.7	202.3	20.2	31.7	4.99	20.50	20.14	.....
4.	Commercial dust preparation B	133.3	7.48	857.3	227.7	.....	27.8	4.83	20.09	20.50	4.67
5.	Commercial dust preparation C	135.1	9.11	938.9	202.7	.....	32.2	4.20	21.14	19.37	.....
6.	1% naphthaleneacetic acid in dust	135.1	.....	.....	233.6	.....	.....	.....	.....	.....	.....
7.	Soaked in water 4 hours	121.5	7.57	.....	.....	.....	.....	.....	.....	.....	.....
8.	Soaked 4 hours in 0.1% naphthaleneacetic acid solution	137.8	0.00	.....	.....	.....	.....	.....	.....	.....	.....
9.	Soaked in water 14 hours	132.9	8.61	.....	.....	.....	.....	.....	.....	.....	4.40
10.	Soaked in 0.01% indolebutyric acid solution 14 hours	127.9	7.07	.....	.....	.....	.....	.....	.....	.....	4.36
11.	Soaked in 0.1% naphthaleneacetic acid solution 14 hours	.....	.....	.....	.....	.....	.....	.....	.....	.....	4.16

* Weight per plot instead of per plant.

TABLE 9

SEED TREATMENT WITH LEVULINIC ACID ON FIELD CROPS. GROWTH GIVEN AS AVERAGE FRESH WEIGHT OF ENTIRE PLANT

Exp. no.	Seed treatment	Turnip (gm.)	Sugar beet (gm.)	Carrot (gm.)	Radish (gm.)	Radish (Wisconsin) (gm.)	Squash (kg.)	Sugar beet* (Wisconsin) (kg.)	Soybean* (kg.)	Buckwheat* (kg.)	Oat* (tops only) (kg.)
1.	Untreated	232.2	957.0	126.1	8.21	32.3	4.581	19.9	19.68	19.59	4.61
2.	Soaked 4 hours in water	198.0	902.0	121.5	7.57	.....	.....	.....	21.64	.....	.....
3.	Soaked 4 hours in 0.1% levulinic acid	222.7	870.0	117.9	6.16	31.3	.....	.....	21.00	.....	.....
4.	Soaked 4 hours in 1% levulinic acid	.....	834.0	.....	.....	.....	4.65	.....	.....	.....	.....
5.	Soaked 14 hours in water	.....	.....	.....	.....	.....	.....	.....	.....	.....	4.40
6.	Soaked 14 hours in 0.1% levulinic acid	.....	.....	.....	.....	.....	.....	20.2	.....	19.19	.....
7.	Soaked 14 hours in 1% levulinic acid	.....	.....	.....	.....	.....	.....	.....	.....	.....	4.72
8.	Dusted with levulinic acid in talc, 1,000 p.p.m.	219.5	848.2	.....	.....	.....	.....	18.6	19.10	.....	.....
9.	Dusted with levulinic acid in talc, 10,000 p.p.m	229.6	961.6	.....	.....	32.65	3.647	.....	21.00	19.19	.....
10.	Dusted with levulinic acid in talc, 1000,000 p.p.m.	229.5	.....	.....	.....	.....	.....	.....	19.27	.....	.....

* Weight per plot instead of per plant.

preparations. Field experiments on sugar beet and radish were duplicated at Lake Geneva, Wisconsin, on a different soil and with different climatic conditions but with similar results.

talc; by dusting with 1 per cent levulinic acid in talc; by soaking 14 hours in 1 per cent levulinic acid; and with three different commercial preparations. The seeds were grown on both fertilized and unfer-

TABLE 10

EFFECT OF SEED TREATMENT WITH GROWTH-REGULATING SUBSTANCES ON U.S. HYBRID 113 AND YELLOW DENT CORN GROWN ON FERTILIZED AND UNFERTILIZED SOIL
FIGURES GIVEN AS AVERAGES

Variety	Soil	Seed treatment*	Fodder			Grain			
			Fresh weight per plot (kg.)	Dry weight per plot (kg.)	No. ears per plot	No. nubbins per plot (gm.)	Fresh weight per ear (gm.)	Dry weight of grain per ear (gm.)	Dry weight of cob per ear (gm.)
U.S. Hybrid	Unfertilized	1. Untreated	25.90	6.53	50.4	3.4	299	161.5	43.4
		2. Commercial preparation A	27.53	6.67	47.5	6.4	317	144.7	38.3
		3. Commercial preparation B	25.63	6.21	45.2	6.4	298	155.8	44.1
		4. Commercial preparation C	26.53	6.71	45.2	5.2	319	165.3	42.5
		5. Dusted 1% naphthaleneacetic acid	28.12	5.90	46.7	6.5	294	162.0	45.0
		6. Dusted 1% levulinic acid	25.49	5.81	49.0	3.0	310	146.0	37.4
		7. Soaked 14 hours in 1% levulinic acid	25.58	6.26	41.2	8.0	294	156.6	43.5
	Fertilized	1. Untreated	30.25	6.08	45.1	7.4	342	191.1	47.7
		2. Commercial preparation A	30.39	5.62	47.6	6.0	340	185.8	45.2
		3. Commercial preparation B	29.66	5.94	48.8	6.1	341	185.4	46.5
		4. Commercial preparation C	30.75	6.35	51.6	5.8	339	185.2	46.9
		5. Dusted 1% naphthaleneacetic acid	29.53	6.12	47.8	4.4	331	187.7	44.9
		6. Dusted 1% levulinic acid	31.16	6.17	50.0	5.3	349	196.0	46.9
		7. Soaked 14 hours in 1% levulinic acid	30.48	5.99	49.3	6.6	346	202.7	41.9
Yellow Dent	Unfertilized	1. Untreated	26.81	7.17	23.0	9.5	322	142.6	41.6
		2. Commercial preparation A	29.53	7.35	24.5	9.8	317	152.8	46.9
		3. Commercial preparation B	30.57	7.94	24.8	9.2	345	135.6	33.2
		4. Commercial preparation C	30.89	7.89	23.0	7.0	351	138.4	42.1
		5. Dusted 1% naphthaleneacetic acid	27.76	7.53	22.4	6.2	305	144.0	39.9
		6. Dusted 1% levulinic acid	29.12	7.39	22.0	10.0	311	128.5	39.3
		7. Soaked 14 hours in 1% levulinic acid	26.04	7.32	17.2	8.2	366	151.6	46.4
	Fertilized	1. Untreated	31.75	7.98	27.5	9.8	308	162.2	45.0
		2. Commercial preparation A	30.71	7.26	23.5	7.0	360	163.8	43.9
		3. Commercial preparation B	31.52	7.08	23.7	7.0	322	169.3	43.4
		4. Commercial preparation C	31.34	7.30	23.8	10.7	345	162.2	43.6
		5. Dusted 1% naphthaleneacetic acid	33.79	8.75	24.7	11.4	344	173.4	51.5
		6. Dusted 1% levulinic acid	34.29	8.25	24.8	7.1	342	171.8	43.5
		7. Soaked 14 hours in 1% levulinic acid	33.11	8.48	28.0	9.4	339	170.3	44.7

* Analyses of variance were made on treatments which showed an increase over the untreated. None of the differences was significant at the 5% level.

3. Levulinic-acid treatments applied in a number of ways to seeds of these crops likewise failed to increase their growth.

4. Seeds of U.S. Hybrid 113 and Yellow Dent corn were treated by dusting with 1 per cent naphthaleneacetic acid in tilized soil. Measurement of stalk and grain yield failed to indicate any significant increase as a result of any of the treatments.

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LITERATURE CITED

1. AMLONG, H. U., and NAUNDORF, G., Wuchsstoffe und Pflanzenertrag. Forschungsdienst. 7: 465–482. 1939.
2. CHOLODNY, N. G., Growth hormones and development of plants. Nature 13:586. 1936.
3. CROXALL, H. C., and OGILVIE, L., The incorporation of growth hormones in seed dressings. Jour. Pomol. 17:362–384. 1940.
4. DEXTER, S. T., Preliminary findings on the use of plant hormones as seed treatments for sugar beets. Mich. Agr. Exp. Sta. Quarterly Bull. 24:245–248. 1942.
5. GRACE, N. H., Physiological curve of response to phytohormones by seeds, growing plants, cuttings, and lower plant forms. Canad. Jour. Res. 15:538–546. 1937.
6. ———, Effect of phytohormones on seeds damaged by formaldehyde and other disinfectants. Canad. Jour. Res. 16:313–329. 1938.
7. ———, Effects of phytohormone, potassium nitrate, and ethyl mercuric bromide on the germination and early growth of wheat. Canad. Jour. Res. 19:211–215. 1941.
8. HOPKINS, J. W., Effect of phytohormone dust seed treatment on growth and yield of barley under greenhouse conditions. Canad. Jour. Res. 18:507–512. 1940.
9. HWANG, Y., and PEARSE, H. L., The response of seeds and seedlings to treatment with indolylacetic acid. Ann. Bot. 4:31–37. 1940.
10. IRELAND, J. C., Treating seed with hormones. Seed World 51:16–19. 1942.
11. JONES, F. D., Hormone treatment of grass seed. National Seedsman 5:42–43. 1939.
12. LAFFERTY, H. A., Effect of certain hormones on barley. Proc. Inter. Seed Test Assoc. 12:19–21. 1940.
13. MCEVOY, E. T., Responses of tobacco seedlings to chemical growth substances. Sci. Agr. 22: 528–532. 1942.
14. MELL, C. W., Treating grass and clover with rootone. Seed World 50:42–44. 1941.
15. MITCHELL, J. W., Effect of indoleacetic acid on the growth of some crop plants. Proc. Amer. Soc. Hort. Sci. 36:171–176. 1938.
16. NUCKOLS, S. B., Plant hormone treatment of sugar beet seed. Sugar Beet Jour. 7:210–211. 1942.
17. STIER, H. L., and DU BUY, H. G., The influence of certain phytohormone treatments on the time of flowering and fruit production of tomato plants under field conditions. Amer. Soc. Hort. Sci. 36:723–731. 1938.
18. TEMPLEMAN, W. G., The effect of some plant growth substances on dry-matter production in plants. Emp. Jour. Exp. Agr. 7:76–88. 1939.
19. TEMPLEMAN, W. G., and MARMOY, C. S., Effect upon the growth of plants of watering with solutions of plant growth substances and of seed dressings containing these materials. Ann. Appl. Biol. 27:453–471. 1940.
20. THIMANN, K. V., and LANE, R. H., After-effects of the treatment of seed with auxin. Amer. Jour. Bot. 25:535–543. 1938.

CHEMICAL CHANGES ACCOMPANYING GROWTH AND DEVELOPMENT OF SEED AND FRUIT OF THE ELBERTA PEACH[1]

F. A. LEE AND H. B. TUKEY

(WITH ONE FIGURE)

Introduction

The peach fruit develops in three stages (1, 2). In stage I the pericarp of the Elberta variety increases in size for a period of about 49 days following full bloom. During stage II it makes only retarded development, at the same time that the embryo reaches nearly maximum size. During stage III, known as the "final swell," the fleshy pericarp increases rapidly in size to fruit ripening.

The present study was undertaken to determine the gross chemical changes that take place under natural growing conditions during this development, both as regards the fleshy and the stony portions of the pericarp, and also the integuments, nucellus, endosperm, and embryo, in so far as possible. This paper is a supplement to a previous one (2) in which chemical changes taking place under artificial conditions of ringing and defoliation were studied. In both papers, special emphasis has been placed on the development of the embryo in relation to the development of the fruit.

Experimental procedure

Studies were conducted during the growing seasons of 1935, 1936, and 1937, in the varietal orchards of the New York State Agricultural Experiment Station and in the commercial orchard of Willis Henderson at Dresden, New York. These were good crop years, providing excellent growing conditions for the samples collected.

Measurements of development of the embryo, endosperm, nucellus, integuments, stony pericarp, and fleshy pericarp were recorded at fortnightly intervals, from which the correlations with the chemical analyses were made.

Chemical analyses were made of fleshy pericarp, stony pericarp, integuments, nucellus, endosperm, and embryo, in so far as possible. Early in stage I the embryo is microscopic, and quantities sufficient for chemical analysis could not be secured. During the early part of stage I also it was not possible to make a clean separation of fleshy from stony pericarp, and so for one sampling these two tissues were taken together. Again, during the early part of stage II it was not practicable to separate nucellus and endosperm, and so these tissues were taken together for one sampling. Partially to compensate for this situation, analyses were made of the entire seed during stage I and early stage II, offering the possibility of arriving at values for some of these tissues by subtraction.

The samples were weighed immediately after their separation and divided into four duplicate lots for determinations. Speed in the preparation of samples was found essential to prevent loss of moisture and also to reduce possible chemical changes. It was necessary to employ

[1] Journal Article no. 468 of the New York State Agricultural Experiment Station.

TABLE 1

CHEMICAL ANALYSES OF TISSUES OF FRUIT AND SEED OF ELBERTA PEACH FROM EARLY STAGES TO FRUIT MATURITY (PERCENTAGE FRESH-WEIGHT BASIS)

Stage of fruit development	Date	Percentage					
		Moisture	Reducing substances calculated as glucose	Sucrose	Ether extract	Nitrogenous substances (N×6.25)	Ash
		Stony and fleshy pericarp*					
I	June 11	85.68	4.02	0.50	0.91	2.15	.....
		Fleshy pericarp					
I	June 22	86.30	4.33	0.46	0.68	1.56	0.41
II	July 8	84.92	4.26	1.35	0.39	1.39	0.38
II	July 21	84.80	3.81	1.43	0.65	1.33	0.56
II	Aug. 3	85.85	3.64	1.83	0.52	1.75	0.54
III	Aug. 19	86.32	4.24	1.86	0.26	1.14	0.61
III	Sep. 11	88.46	3.53	3.37	0.14	0.82	0.32
III	Sep. 27	87.05	2.94	6.93	0.15	0.83	0.34
		Stony pericarp					
I	June 11	.....	.....	.....	.....	.....	.....
I	June 22	89.74	4.71	0.21	0.55	1.52	0.31
II	July 8	84.71	3.74	0.58	0.52	1.71	0.45
II	July 21	56.52	1.60	0.38	0.33	2.04	0.44
II	Aug. 3	42.35	0.77	0.25	0.22	2.26	0.32
III	Aug. 19	30.04	0.64	0.11	0.18	1.15	0.21
III	Sep. 11	24.71	0.35	0.12	0.11	0.67	0.15
III	Sep. 27	23.47	0.39	0.13	0.13	0.65	0.13
		Entire seed					
I	June 11	91.73	0.94	0.37	0.75	3.13	.....
I	June 22	93.20	0.93	0.28	0.51	2.28	0.66
II	July 8	93.05	0.49	0.17	0.58	2.56	0.56
		Integument					
I	June 22	88.07	1.33	0.44	0.70	4.11	.....
II	July 8	87.75	0.55	0.20	0.67	4.96	0.97
II	July 21	83.18	0.89	0.33	0.54	4.11	1.03
II	Aug. 3	76.00	1.39	1.33	1.70	5.03	1.26
III	Aug. 19	73.15	1.78	1.94	3.17	4.43	1.10
III	Sep. 11	65.20	1.64	0.27	6.42	4.44	1.03
III	Sep. 27	47.45	2.06	0.57	9.64	7.04	1.34

* Tissues not sufficiently defined to separate in this one sample.

TABLE 1—*Continued*

Stage of fruit development	Date	Percentage					
		Moisture	Reducing substances calculated as glucose	Sucrose	Ether extract	Nitrogenous substances (N×6.25)	Ash
		Nucellus and endosperm					
II.....	July 21	94.86	0.47	0.13	0.25	2.97	0.41
		Embryo					
II.....	July 21	92.62	0.57	1.04	0.34	3.98	0.57
II.....	Aug. 3	86.45	0.35	0.50	2.52	8.27	0.76
III.....	Aug. 19	66.40	0.75	1.06	11.70	13.95	1.53
III.....	Sep. 11	42.47	0.71	1.40	29.14	16.65	1.94
III.....	Sep. 27	40.68	0.37	2.18	29.40	19.47	1.92

five to ten persons, working rapidly, particularly for the preparation of the samples of the smaller tissues, as integuments.

Determinations were made for moisture, reducing substances as glucose, sucrose, ether extract (fat), total nitrogen, and ash (mineral matter). The reducing substances calculated as glucose were further treated with Fleischmann's starch-free yeast to determine the proportion fermentable with yeast.

The samples for fat were dried at 95° C. for 2 hours and then placed in a vacuum at 70° until they reached constant weight. The percentage of moisture was determined from these samples. The samples for sugar were placed directly into 80 per cent alcohol and boiled slowly for 15 minutes before setting away. During the analyses for sugars these substances were extracted by means of 80 per cent alcohol, in the usual way (4). The acid in the samples of fleshy pericarp was neutralized with calcium carbonate before heating. The Munson-Walker method by direct weighing was used for all sugars. Sucrose was inverted by means of invertase, as was done in the analyses presented previously (2, 3). For nitrogen, the Gunning method was used (4), and fat was determined by the usual Soxhlet extraction method (4).

Calculations were made both on the unit-weight basis and in percentage based upon fresh weight.

Results

Although analyses were made of material for the seasons of 1935, 1936, and 1937, only those for 1937 are presented here, inasmuch as the data from the 1935 and 1936 series closely approximate those for 1937, and the latter are more complete. Mineral matter, however, was determined only for the 1937 series. The data are given on the percentage fresh-weight basis in table 1 and on the unit fresh-weight basis in table 2.

MOISTURE.—The moisture content of the fleshy pericarp on the unit basis was found to follow the general curve for

TABLE 2

CHEMICAL ANALYSES OF TISSUES OF FRUIT AND SEED OF ELBERTA PEACH FROM EARLY STAGES TO FRUIT MATURITY (UNIT-WEIGHT BASIS [MG.] OF FRESH MATERIAL)

Stage of fruit development	Date	Milligrams						
		Unit weight	Moisture	Reducing substances calculated as glucose	Sucrose	Ether extract	Nitrogenous substances (N×6.25)	Ash
		Fleshy and stony pericarp						
I.....	June 11	2499.7	2149.5	100.4	12.5	24.7	53.6	.....
		Fleshy pericarp*						
I.....	June 22	5757.0	4968.0	259.3	26.5	39.7	89.9	28.6
II.....	July 8	13615.7	11555.0	580.0	183.8	55.3	189.1	52.0
II.....	July 21	15810.3	13410.0	606.0	227.4	102.8	210.7	88.6
II.....	Aug. 3	16746.5	14385.0	610.0	306.3	87.1	292.9	90.4
III.....	Aug. 19	29435.0	25430.0	1248.3	547.2	76.6	335.8	179.6
III.....	Sep. 11	64573.4	57200.0	2272.0	2175.0	94.0	532.1	206.8
III.....	Sep. 27	75645.0	65875.0	2408.0	5059.0	113.6	628.1	257.3
		Stony pericarp						
I.....	June 11	.....	.....	.....	.....	.....	.....	.....
I.....	June 22	2760.0	2477.0	130.0	5.8	15.7	42.0	7.1
II.....	July 8	4708.3	3995.0	176.1	27.3	25.9	80.2	21.2
II.....	July 21	5135.0	2901.0	82.2	19.5	16.9	104.6	22.6
II.....	Aug. 3	5087.4	2157.0	39.3	12.7	11.2	115.1	16.3
III.....	Aug. 19	5432.5	1634.0	34.8	6.0	9.8	62.5	11.4
III.....	Sep. 11	5990.3	1344.0	21.0	7.2	6.0	40.3	9.0
III.....	Sep. 27	5237.5	1229.0	20.4	6.8	6.8	34.6	6.8
		Entire seed						
I.....	June 11	83.9	76.9	0.8	0.3	0.6	2.6	.....
I.....	June 22	380.7	355.4	3.5	1.7	1.9	8.7	2.5
II.....	July 8	647.4	602.0	3.2	1.1	3.0	16.6	3.6
		Integument						
I.....	June 22	97.0	85.6	1.3	0.4	0.7	4.0	.....
II.....	July 8	196.5	170.4	1.1	0.4	1.2	9.8	1.9
II.....	July 21	229.0	190.5	2.0	0.8	1.2	9.4	2.4
II.....	Aug. 3	151.6	115.3	2.1	2.0	2.6	7.6	1.9
III.....	Aug. 19	124.5	91.2	2.2	2.4	4.0	5.5	1.4
III.....	Sep. 11	86.8	81.2	1.4	0.2	8.0	3.9	0.9
III.....	Sep. 27	51.1	24.3	1.1	0.3	4.9	3.6	0.7

* Tissues not sufficiently defined to separate in this one sample.

TABLE 2—*Continued*

Stage of fruit development	Date	Milligrams						
		Unit weight	Moisture	Reducing substances calculated as glucose	Sucrose	Ether extract	Nitrogenous substances (N×6.25)	Ash
		Nucellus and endosperm						
II.....	July 21	177.7	168.6	0.8	0.2	0.4	5.1	0.8
		Embryo						
II.....	July 21	214.1	198.4	1.2	2.3	0.7	8.5	1.2
II.....	Aug. 3	511.4	442.3	1.8	2.6	12.9	42.3	3.9
III.....	Aug. 19	537.8	357.2	4.0	5.7	62.9	74.9	8.6
III.....	Sep. 11	565.3	228.1	4.0	7.9	156.6	95.3	11.0
III.....	Sep. 27	574.8	234.1	2.1	12.5	169.2	111.9	11.1

growth of the fruit. During stage I it increased more than five times; during stage II it increased only slightly; and during stage III it increased markedly to more than five times that at the beginning of stage III. The actual percentage of moisture remained at a fairly constant level throughout all stages, however, with some increase toward the end of stage III.

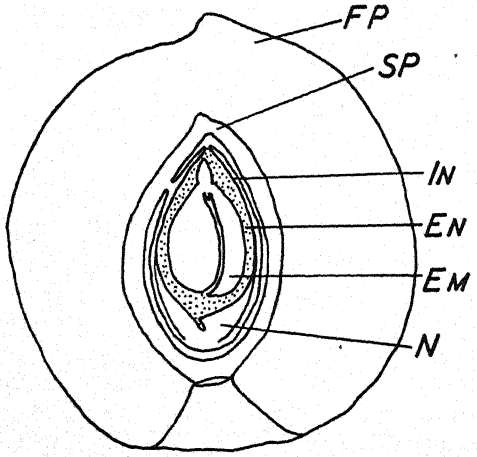


FIG. 1.—Longitudinal section of developing peach showing parts included in chemical analyses. *fp*, fleshy pericarp; *sp*, stony pericarp; *in*, integuments; *n*, nucellus; *en*, endosperm; and *em*, embryo.

Moisture in the stony pericarp decreased progressively with advancing age of the material on the percentage basis, but on the unit basis there was an increase in moisture during stage I accompanying increase in size, followed by a steady decrease during stages II and III, accompanying differentiation and hardening. The significance of these results is treated more fully in a previous paper (2), in which the role the stony pericarp may play in embryo development is discussed.

On the percentage basis the moisture content of the integuments became progressively lower, especially during stage III, while on the unit basis it showed increases up to the middle of stage II, followed by gradual decreases thereafter, accompanying drying out and maturing of the tissues. The percentage moisture in the embryo showed a steady decline,

from the time in stage II when samples could be secured until the end of stage III; while on the unit basis there was marked increase in moisture during stage II, followed by decline during stage III accompanying the differentiation of tissues and the accumulation of storage materials.

ETHER EXTRACT (FAT).—The amount of ether extract (fat) by both systems of calculation was irregular for the fleshy pericarp. On the percentage basis it declined during stage I, increased by a small degree during stage II, and decreased further to a constant level by the middle of stage III. On the unit basis it increased during stage I and the first part of stage II, then declined until the middle of stage III, when it again increased until fruit ripening. The stony pericarp showed progressive decrease in ether extract on the basis of percentage throughout all three stages. On the unit basis it showed an increase during stage I, followed by a progressive decrease during stages II and III. For the integuments, on the percentage basis it declined slightly but consistently until toward the end of stage II, when it increased steadily and appreciably to fruit ripening. On the unit basis it increased slowly during stages I and II, increased rapidly during the first part of stage III, and then declined toward the end of that stage. The embryo, which contains a relatively high percentage of ether extract at maturity, showed increase during all three stages, both on the percentage basis and the unit basis.

REDUCING SUBSTANCES CALCULATED AS GLUCOSE.—Reducing substances calculated as glucose require special mention because aliquots of each sample were treated with starch-free yeast and reducing substances again determined. In all instances all except traces of these materials were fermented, indicating that these substances are definitely reducing sugars.

On the unit-weight basis reducing substances accumulated steadily in the fleshy pericarp during stage I, slightly during stage II, and markedly during stage III. On the percentage basis they were irregular, remaining fairly constant during stage I, declining slightly during stage II, increasing during the first part of stage III, and then declining as the end of that stage approached. With the stony pericarp, on the unit basis they were relatively high during stage I, increasing still further during the period, but declining steadily during stages II and III at a time when this tissue was differentiating and hardening. On the percentage basis the decline was steady during all three stages to fruit ripening. With the integuments, the results were variable for both methods of expression. On the percentage basis they showed a decline from stage I to stage II, an increase during stage II, a decline during the first part of stage II, followed by an increase toward the end of stage III. On the unit basis there was a slight decline from stage I to stage II, a slight increase during stage II, and a decline during stage III. For the embryo, on the percentage basis they declined while the embryo was making rapid increase during stage II, then increased as storage materials accumulated at the beginning of stage III, and declined toward the end of stage III. On the unit basis they increased slightly during stage II, sharply during the first part of stage III, and then dropped sharply as fruit ripening approached.

SUCROSE.—Sucrose increased steadily though slowly during stages I and II, as calculated on the unit basis, and increased greatly during stage III to more than fifteen times what it was at the be-

ginning of that stage. When calculated on the percentage basis the sucrose content increased slowly at first, followed by rapid increase. In the stony pericarp the trend is similar for both methods of expression, namely, increase during stage I followed by steady decline during stage II to a low level, which was maintained at that point throughout stage III. The integuments showed a somewhat similar trend to that for the stony pericarp by both methods of calculation. For the embryo, there was a slight increase in sucrose on the unit basis during stage II, and marked and rapid increase during stage III. On the percentage basis there was slight decline during stage II and increase during stage III.

NITROGENOUS SUBSTANCES.—Nitrogenous substances ($N \times 6.25$) declined rather steadily for the fleshy pericarp during all three stages on the percentage basis, while on the unit basis the reverse was true. The increase in nitrogenous substances was particularly rapid and great in amount during stage III. For the stony pericarp, on both the unit and percentage basis there was an increase during stage I, slight increase during stage II, and a sharp and steady decline during stage III to fruit ripening. The integuments showed an increase on the unit basis during stage I, followed by a decline during the last of stage II and throughout stage III as the tissue differentiated. On the percentage basis it remained at a fairly constant level until the end of stage III, when it increased as the tissues lost moisture. The embryo showed substantial increases by both methods of expression throughout all three stages.

STARCH.—Qualitative tests, as reported previously (2), showed starch negative in the embryo, stony pericarp, and ripe fleshy pericarp, although small amounts were found in the fleshy pericarp in early stages of fruit development when the fruit was green.

ASH (MINERALS).—The mineral constituents, as represented by ash content, increased steadily in the fleshy pericarp on the unit basis. On the percentage basis they increased slightly until the beginning of stage III and then sharply declined. In the stony pericarp, on both the unit and percentage basis the amount increased during stage I, began to decline toward the end of stage II, and continued steady decline until fruit ripening. In the integuments the same relation prevailed on the unit basis, but on the percentage basis the ash increased during stage II, declined slightly during the first part of stage III, and increased as the end of the stage was reached. In the embryo, by both methods of calculation the ash content increased steadily during stage II and the first part of stage III, and remained at that point until fruit ripening.

Discussion

The general pattern for chemical composition of fruit and seed parts of the peach during development is similar to that for much plant material. Especially is this true when the data are correlated with morphological development.

For example, during the first part of stage I, when active growth is taking place in the fleshy and stony pericarp and nucellus and integuments, and at a time when some of this growth is by cell division, the percentage moisture is relatively high and such labile forms as reducing sugars are also fairly high in amount. During stage II, when little increase in size occurs in the fleshy portion of the pericarp, changes in composition are small. It is during this period, however, that thickening and hardening of the cell walls of the stony portion of the pericarp

begin, so that there is steady decline in percentage moisture, reducing sugars, sucrose, ether extract, and ash in that portion.

Further, active growth of the embryo begins during this stage, correlated with high moisture level and low content of such storage forms as ether extract and nitrogenous substances. During stage III, when increase in size of the fleshy pericarp is rapid, caused largely by cell expansion, the sucrose content increases markedly and the moisture content slightly; while the percentages of reducing substances, ether extract, nitrogenous substances, and ash decline. On the other hand, since the cell walls of the stony pericarp thicken and harden still further during this period, and since it is not an organ for the storage of such materials as were included in the analyses, the percentages of moisture, reducing substances, sucrose, ether extract, nitrogenous substances, and ash decline to the lowest level observed during development. At the same time, in the embryo, which at fruit ripening contains a high percentage of such storage forms as ether extract and nitrogenous substances, rapid accumulation of these materials continues while the moisture declines.

The data show especially well in this way the necessity for careful separation of plant tissues and parts intended for chemical analyses, rather than the combining of related parts, as has so frequently been done. A final example is shown in the development of the seed. During stage I it consists almost entirely of nucellus and integument, which have high moisture content. As development proceeds the integuments lose moisture, the nucellar tissue is digested by the endosperm, and the endosperm is in turn digested and replaced by the embryo—which finally accumulates storage materials in quantity. Accordingly, analyses of the total seed at different periods in development are really analyses of widely different tissues.

Summary

1. The moisture content, reducing substances calculated as glucose, sucrose, ether extract (fat), total nitrogen, and ash (minerals), are given for the fleshy pericarp, stony pericarp, integument, nucellus and endosperm, and embryo at regular intervals during the growing season at such times as the parts analyzed were of sufficient size to permit adequate sampling.

2. The analyses are correlated with morphological development of the plant parts analyzed.

3. The data show the importance of careful separation of tissues intended for analyses and the value of considering the morphological nature of developing plant parts in connection with such analyses.

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LITERATURE CITED

1. Tukey, H. B., Growth of the peach embryo in relation to growth of fruit and season of ripening. Proc. Amer. Soc. Hort. Sci. 30:209–218. 1933.
2. Tukey, H. B., and Lee, F. A., Growth and development of the embryo and fruit of the peach as affected by ringing and defoliation of the branches. Bot. Gaz. 101:818–838. 1940.
3. ———, and ———, Embryo abortion in the peach in relation to chemical composition and season of fruit ripening. Bot. Gaz. 98:586–596. 1937.
4. Association of Official Agricultural Chemists. Methods of analysis. 5th ed. 1940.

HISTOLOGICAL STUDY OF FREEZING, DESICCATION, AND WINTER INJURY OF RASPBERRY CANES[1]

AUSTIN O. SIMONDS

(WITH EIGHT FIGURES)

Introduction

Winter injury to many varieties of raspberries is of common occurrence in northern Colorado, and is attributed to a number of causes. Prominent among the causes are the breaking of certain tissues subsequent to the formation of ice crystals, protoplasmic death resulting directly from low temperatures, and injury of the protoplasm owing to extreme desiccation. In this latter regard, BRIERLEY[2] reports that "it is probable that many cases of winter injury reported for otherwise hardy raspberries have been due to desiccation." He found that winter injury occurred when the moisture content of the plant fell below 50 per cent of the dry weight and considered that this condition was brought about when deep freezing of the soil interfered with normal conduction in the canes.

A study was made of normal and injured raspberry canes in order to determine the types of tissue injuries resulting from apparent winter injury in the field, and to compare these with artificially induced injuries resulting from both freezing temperatures and slow desiccation at temperatures above freezing.

[1] Published with the approval of the Director as Paper no. 154, Scientific Journal Series, Colorado Agricultural Experiment Station.

[2] BRIERLEY, W. B., Desiccation in the Latham raspberry. Amer. Soc. Hort. Sci. Proc. 31:Supl. pp. 110–113. 1934.

Material and methods

FIELD STUDIES

Apparently uninjured Latham raspberry canes were obtained at random from field plantings of the Horticulture Section, Colorado Agricultural Experiment Station, on March 17, 1938. A second collection was made from the same field on June 27, after evidence of winter injury was apparent. Samples from these collections were cut into small pieces with a fine-toothed electric rotary saw, prior to placing them in the fixing agent. This method prevented crushing of the outer stem tissues. The teeth of the saw produced a shallow roughened end, which was discarded at the time of sectioning.

The material was fixed in formalin-acetic alcohol (7 cc. formalin, 5 cc. glacial acetic acid, and 88 cc. of 50 per cent ethyl alcohol). A preliminary treatment of 1–15 minutes in a solution containing 0.5–1 per cent sodium hypochlorite was found desirable to remove the brown tannin-like substance present in the phloem and inner cortical cells of mature canes. The required concentration of chlorine in the solution and the length of time necessary to clear the cells of this color varied, apparently depending upon the age of the section and its proximity to the tip of the cane. For purposes of comparison, however, some samples were prepared without use of the hypochlorite solution. Following decolorizing, sam-

ples were treated with hydrofluoric acid, imbedded in celloidin, and cut on a sliding microtome. Other pieces were imbedded without the hydrofluoric-acid treatment (which was found unnecessary), while still others were cut on the microtome without either imbedding or treating with the acid. The best preparations were most consistently obtained from material imbedded in celloidin. Of the several staining combinations used, fast green F.C.F. with safranin O proved most satisfactory and was used most frequently.

Artificially induced injuries

To determine the results of artificially induced freezing and desiccation, six plants averaging four canes each were transplanted to soil in large cans on November 4, 1939. Two of these plants were then subjected to continuous refrigeration at 8° F. for 12 weeks. Two other plants were placed in the greenhouse and allowed to approach air dryness at temperatures above freezing. The remaining two plants were alternated between the refrigerator (8° F.) and the greenhouse (75° F.) at 7-day intervals for the first 8 weeks and at 14-day intervals for the remaining 4 weeks. Water was added only to the plants given the alternating treatment.

Samples were taken at the end of 4 weeks from plants left to desiccate, and at the end of 2, 3, 5, 10, and 12 weeks from those in continuous refrigeration. Samples were taken from plants given the weekly alternating treatment as follows: 1-week refrigeration and 1-week greenhouse; 2-weeks refrigeration and 1-week greenhouse; 3-weeks refrigeration and 2-weeks greenhouse; 5-weeks refrigeration and 5-weeks greenhouse; and 6-weeks refrigeration and 6-weeks greenhouse. The same methods of preparation were used as described for field material.

Further studies of the effect of freezing were conducted by taking 6-inch pieces of green plants from the field on July 24, August 1, and September 19, 1940, and freezing them in the refrigerator at 23° F. for 1 week before making sections. Samples taken from the middle of these pieces were immediately cut into sections without imbedding. These sections were fixed in 50 per cent ethyl alcohol for 2 hours before being treated with the sodium-hypochlorite solution and before being stained. Other pieces were placed in the formalin-acetic-alcohol fixing agent and sectioned the following day.

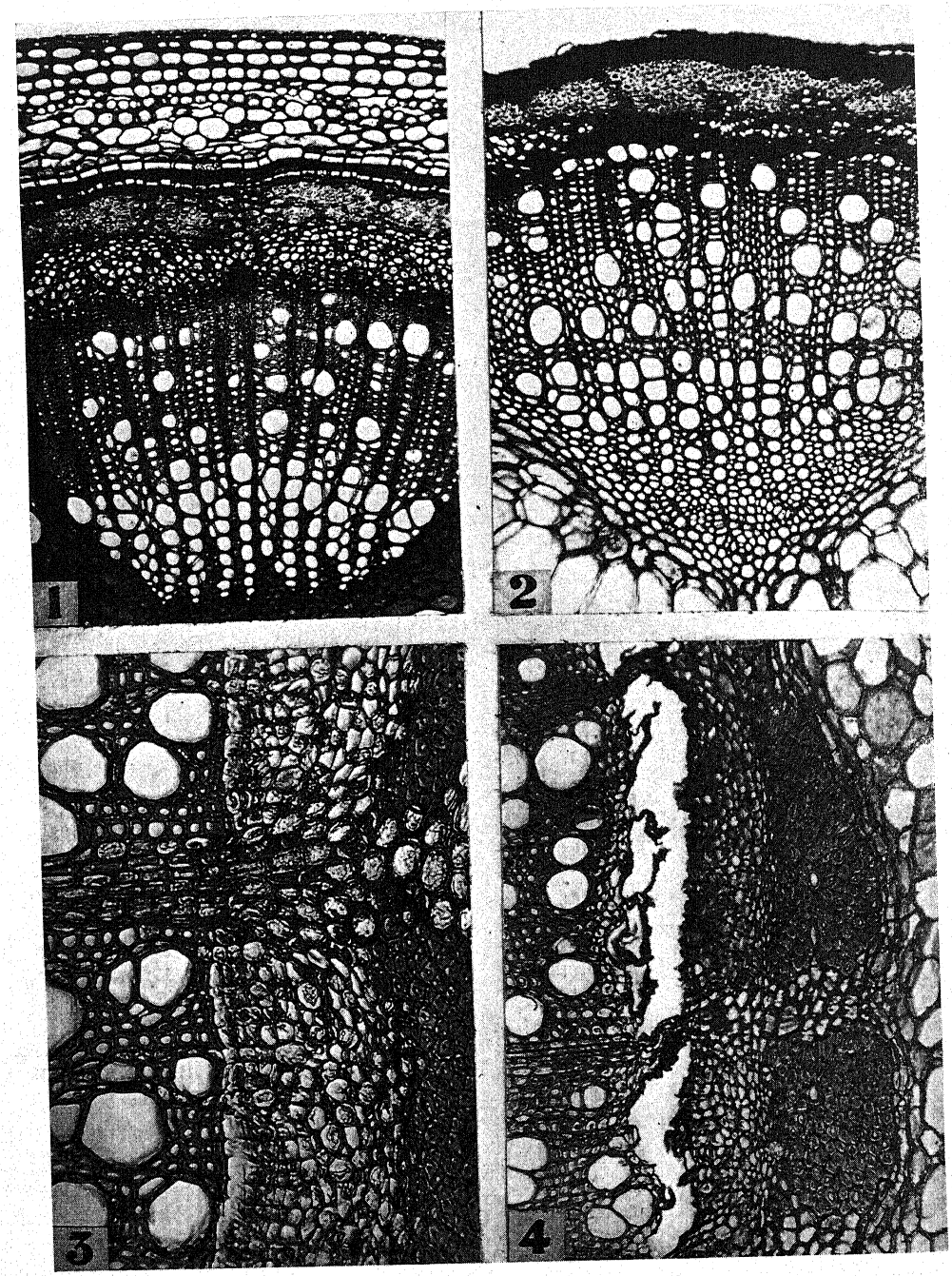
Observations

Naturally occurring field injuries

Many samples collected in 1938 showed no abnormal conditions in the tissues of the stem. However, some sections of canes did show a distinct breaking of cell walls in the outer tissues. These breaks were of a nature that precluded the possibility of mechanical injuries in the process of cutting the sections.

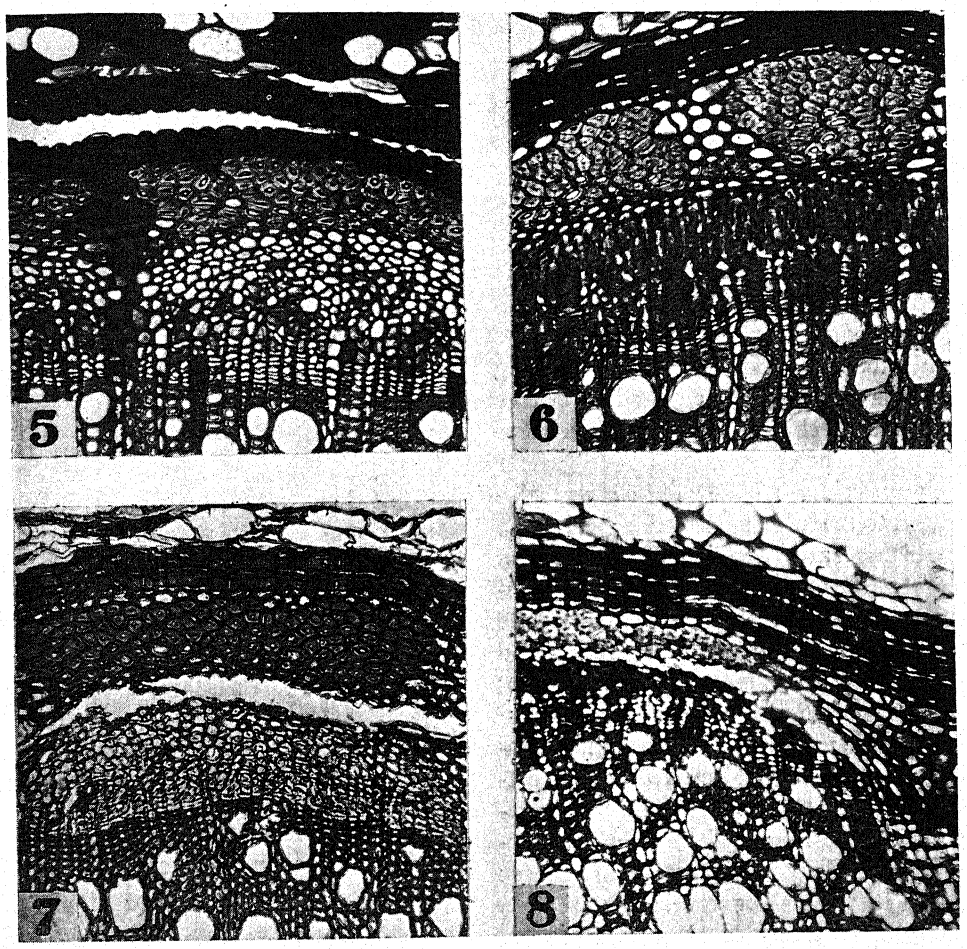
One type of injury was the presence of broken cambial cells. The rupture occurred in the radial walls of the cells, so that broken areas extended tangentially and parallel to the outermost cells of the secondary xylem (fig. 3). A second type of break was evident in the outer region of the primary phloem adjacent to the pericyclic fibers. The ruptured area in general extended tangentially (fig. 7). Frequently radial breaks were found to extend down to the side of the multiseriate rays. More rarely the tissue of the phloem was split down one of the uniseriate rays near the center of a large bundle.

The outer portions of the cortex and epidermis were frequently found to be



FIGS. 1–4.—Fig. 1, normal cane from field (Oct. 4, 1940). Fig. 2, section from dead cane, field collection, showing tannin-like substance in blackened tissues. Fig. 3, broken cambial layer found in field sample. Fig. 4, broken cells of cambial region produced artificially by freezing green canes.

broken off in flakes. Occasionally such breaks appeared in the small, thin-walled parenchymatous cells just outside the abscission layer.[3] More commonly the separation was found in the thin-walled cells of the abscission layer, formed between two layers of thick-walled lignified cells. This caused the outer tissues to be shed in large flakes (fig. 5). The latter condition was not considered an injury but the normal abscission of the plant tissue. The abscission layer was formed in the cortex just outside the pericycle (fig. 1).



FIGS. 5–8.—Fig, 5, section from field sample (Feb. 2, 1940) showing normal phloem condition with splitting through abscission layer. Fig. 6, necrosis of outer phloem from dormant plants alternated weekly between refrigerator and greenhouse. Fig. 7, broken area in primary phloem from field sample. Fig. 8, artificially produced break in primary phloem in canes refrigerated for 10 weeks continuously.

Rupture of the tissue was not evident in some of the field material, but phloem necrosis was evident as a noticeable breakdown of walls of many sieve tubes. These walls were indistinct, as if partially dissolved, suggesting enzymatic action.

[3] HABERLANDT, G., Physiological plant anatomy. pp. 143–144. 1914.

This injury was usually accompanied by deposition of much brown tannin-like material in all the living cells outside the cambium. The presence of such tannin-like material was also observed in dead canes taken from the field (fig. 2).

Artificially induced injuries

Alternate freezing and thawing did not break dormancy in the transplanted plants, and the ruptured-tissue type of injury was not evident. (Evidence of the failure to break dormancy was in the lack of new cell formation by the cambium and the uniform staining reaction of cells adjacent to the cambium.) Phloem necrosis was most pronounced in the plants subjected to periods of alternate freezing and thawing. Plants exposed to twelve alternate weekly periods in the refrigerator and in the greenhouse showed greater phloem injury (fig. 6) than those subjected to two alternate weekly periods in the refrigerator and in the greenhouse. Plants given treatments intermediate between these extremes (three alternate weekly periods in the refrigerator and two in the greenhouse) showed various results. Some canes from plants given the latter treatment showed no more injury than those subjected to the shortest alternate treatment, while others revealed more injury. Frequently a vascular bundle showing normal phloem was found between the necrotic areas.

Injuries resulting from continuous freezing.—Rupture of the primary phloem cells was found in sections from plants left in continuous refrigeration for 10 and 12 weeks (fig. 8). The breaks appeared to start in cells adjacent to the multiseriate rays and to spread tangentially across the bundles to the opposite sides. Some phloem necrosis was evident but was not so noticeable as in canes given the alternate freezing and thawing treatment.

Fresh green canes with leaves attached were removed from the field in July and August, 1940, and subjected to continuous refrigeration for 1 week (fig. 4). Rupture of the cambial region was then observed. Green samples having leaves, taken from the field September 17, 1940, and subjected to the same treatment, did not show a broken cambium.

Injuries resulting from desiccation.—Plants allowed to dry out exhibited no broken cells. Although the tissues were intact, the sieve-tube contents appeared to be more shrunken than those observed in normal material. Further, in the tissues of desiccated plants the entire region outside the cambium was darker and contained more of the tannin-like material than was observed in the non-desiccated control material. Finally, the break in the abscission layer was more pronounced in the case of the desiccated canes.

Summary

1. Histological studies were made of uninjured and apparently winter-injured raspberry canes in the field. In addition, similar studies of artificially induced injuries were made after subjecting canes to continuous freezing, alternate freezing and thawing, and desiccation above freezing temperatures.

2. Field injuries believed to have been caused by freezing compared favorably with both types of artificially induced freezing injuries. Phloem necrosis, phloem rupture, cambial rupture, and the deposition of tannin-like material in the outer parenchymatous stem tissues were characteristic features of the field material. Injuries due to alternate freezing and thawing were principally char-

acterized by phloem necrosis. Canes subjected to continuous refrigeration revealed phloem rupture in dormant canes and ruptured cambial cells in nondormant ones, accompanied by limited phloem necrosis.

3. Field injuries did not show the same type of tissue impairment as found in canes subjected to desiccation above freezing temperatures. The latter type of injury was characterized by marked deposition of the tannin-like material, reduction in size and shrinkage of the contents of all living cells outside the cambium, and pronounced splitting of abscission layers.

The writer is indebted to GEORGE BEACH, Associate Horticulturist, Colorado Agricultural Experiment Station, for calling attention to this problem and furnishing the material.

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EFFECTS OF VAPOROUS NAPHTHOXYACETIC ACID ON DEVELOPMENT OF TOMATO FRUITS, WITH SPECIAL REFERENCE TO THEIR VITAMIN C CONTENT

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(WITH TWO FIGURES)

The use of growth-regulating substances for the purpose of increasing fruit set has recently been suggested as a possible aid in the commercial production of greenhouse tomatoes. Several methods of applying the treatments have been used, including spraying the flowers with emulsions containing the growth substances; smearing the styles with a paste containing them; or applying the substance as a vapor to the buds and newly opened flowers (3, 5, 7). Seedless fruits that result from such treatments are reported to set well and to grow to a slightly larger size than those that develop from untreated flowers (3, 5). The color and flavor of these artificially induced parthenocarpic fruits are comparable with those of field-grown seeded ones.

Since it has been shown that seed development is greatly reduced (2, 5, 6) and the amount of gelatinous pulp increased in tomato fruits as a result of treating the flowers with growth-regulating substances, it was considered of interest to determine whether these anatomical changes in the fruit were associated with noticeable differences in some constituents of nutritional interest. The vitamin C and ash contents of fruits from pollinated flowers were compared with those of fruits from flowers which had been treated with vaporous β-naphthoxyacetic acid just prior to pollination.

[1] Physiologist, [2] Junior Plant Physiologist; U.S. Bureau of Plant Industry Station, Beltsville, Maryland.

Material and methods

Ninety Bonny Best tomato plants of uniform size were grown in a greenhouse in soil contained in 10-inch clay pots. The plants were arranged in rows extending across the benches, a row of treated plants alternating with a row of untreated ones. Three treated and three untreated rows constituted a block, the entire experiment being made up of five blocks distributed throughout the house. In gathering samples for analyses, equal numbers of treated and control fruits were gathered at random from each block.

The most effective time at which to apply the treatment was determined by means of preliminary experiments, which showed that the greatest number of seedless fruits (50–70 per cent) developed when the flowers were treated at the time of pollination or 1–5 days previously. Very few seedless fruits were developed when the treatment was applied 3–7 days after pollination. When the plants of the present experiment had grown until one flower on the first cluster was fully open and a second beginning to open (fig. 1), the plants to be treated were moved from the greenhouse to an adjacent room (approximately 2500 cubic feet capacity)

which could be tightly closed. They were treated by vaporizing β-naphthoxyacetic acid (250 mg./1000 cubic feet) on a hot glass plate and allowing the plants to remain in the closed, vapor-filled room overnight. The plants were then returned to their original positions on the greenhouse benches. An equal number of control plants were allowed to remain in the greenhouse away from the vapor. Each flower of both treated and control plants was carefully pollinated when open and tagged with a record of its stage of development at the time of treatment. The fruits were then allowed to grow and ripen during the months of February, March, and April.

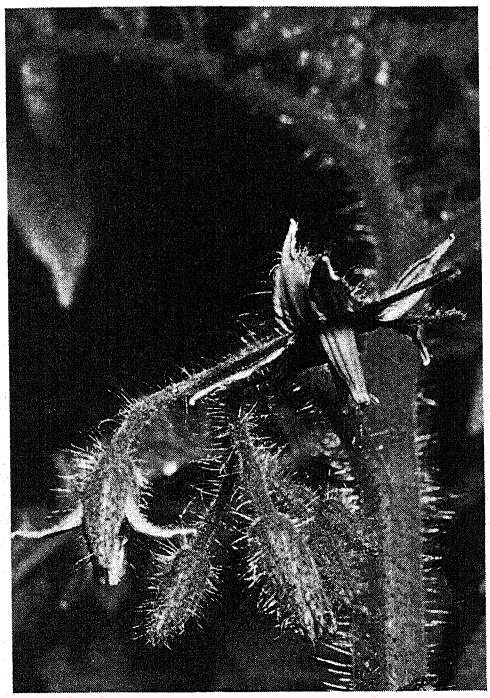


FIG. 1.—Cluster of tomato flowers showing stages of opening when naphthoxyacetic acid vapor was applied.

In another experiment fifty selected Marglobe tomato plants were grown in a greenhouse section with a capacity of approximately 4000 cubic feet. These plants were treated at intervals with vaporous naphthoxyacetic acid as previously described, except that all the plants were left in place and the chemical volatilized in the closed section. Treatment was applied when flowers on the first cluster of a majority of the plants had reached the stage of development shown in figure 1. Identical treatments were given flowers of the second and third clusters when they had reached this stage of development, while the fourth cluster was left untreated. In this experiment none of the flowers were hand pollinated, but some natural pollination doubtless occurred.

The soil in which the plants were grown was frequently fertilized with liquid manure, and the plants grew vigorously during both experiments.

Ripeness was designated as the stage of maturity attained on the day the fruit was completely red in color. Only ripe fruits were collected for determinations of percentage solids and the ash and vitamin C content. The vitamin determinations were made immediately after the fruits were picked by cutting a median slice, approximately 1 cm. in thickness, from each fruit (4). The method used to determine the vitamin content of these sections is described by DAVIS (1).

The percentage of solids and the ash content were determined in aliquots taken from fruits that had been finely ground in a blender. For moisture determinations the samples were dried at 80°–85° C. for approximately 18 hours in a well-ventilated oven, then the residue was ignited in a muffle furnace for ash content.

Results

GUSTAFSON (3) has shown that fruit set on field-grown plants was increased as a result of the application of naphthoxyacetic acid paste to the cut surface of the styles of tomato flowers, and HOWLETT (5) has demonstrated that fruit set in a greenhouse was improved when pollination was supplemented by the application of indolebutyric acid to the severed styles. This effect was especially noticeable when conditions were not favorable for pollination (5).

TABLE 1

RESPONSES OF TOMATO FRUITS OF FIRST CLUSTER TO TREATMENT WITH VAPOROUS NAPHTHOXYACETIC ACID. ASH AND VITAMIN CONTENT EXPRESSED AS AVERAGE OF FIFTEEN DETERMINATIONS

TREATMENT	FRUIT SET*		FRUIT NOT FULLY SEEDED (%)	SEEDLESS FRUIT (%)	AVERAGE FRESH WEIGHT OF RIPE FRUIT (GM.)	ASH (%)	VITAMIN C CONTENT (MG/100 GM. FRESH WEIGHT)
	FIRST TWO BLOSSOMS OPEN (%)	REMAINDER OF BLOSSOMS (%)					
Control..	84	33	9	6	152	0.49	17.7
Treated..	78	45	80	50	152	0.47	18.9

*Differences of 11 required for significance for odds of 19 to 1.

In the present experiment, individual flowers of a cluster responded differently to treatment with respect to the number of fruits set. Somewhat fewer fruits developed from the first two flowers that opened in the first cluster on treated plants than from the first two flowers in a corresponding cluster on untreated plants, but this difference was not statistically significant. On the other hand, treatment significantly increased the number of fruits that developed from the remaining flowers in the first clusters (table 1), although most of these were tightly closed at the time of vaporizing and did not open until several days later. Fruit set on the second clusters was not significantly affected by treatment. The effect on fruit set in the third clusters was not measured, since their flowers were even more immature at the time of treatment than were those on the second clusters.

There was a marked difference in the growth of seeds in treated as compared with untreated fruits of the first cluster. Approximately 80 per cent of the treated ones contained fewer seeds than did the controls, and 50 per cent were entirely seedless. Even more striking results were observed when Marglobe plants were treated repeatedly with vaporous naphthoxyacetic acid. Ninety-eight per cent of the fruits on the first cluster were seedless; 92 and 80 per cent of those on the second and third clusters, respectively, were seedless; while only 6 per cent of those on the untreated fourth cluster were without seeds.

Although the treatment specifically inhibited the growth of seeds, it did not decrease the growth of locule contents other than the seeds, since the number of unfilled locules in either treated or untreated fruits was negligible (fig. 2). HOWLETT (5) reported that the use of indolebutyric acid also inhibited seed growth of greenhouse tomatoes and in some instances even increased the amount of gelatinous pulp.

Although treatment with naphthoxyacetic acid specifically inhibited growth and development of seeds of the Bonny Best variety, the vitamin C or ash contents of the fruits were not significantly altered as a result of treatment (table 1). There was no significant difference between the size, fresh weight, or percentage of solids in treated and control fruits, neither was there a noticeable difference in their color or flavor when ripe.

Summary

1. In experiments with tomato, the use of vaporized β-naphthoxyacetic acid under greenhouse conditions resulted in the development of a high percentage of seedless fruits (50–98 per cent). With the Marglobe variety the response was readily obtained by fumigating the plants in position when the earliest flowers of each successive cluster opened. This method, although causing temporary epinasty to a slight degree, did not appreciably affect vegetative growth of the plants. With Bonny Best variety slight increase in fruit set was observed, and this effect was limited to flowers of the cluster which were well developed but not yet open at the time of treatment.

2. With respect to vitamin C content, the nutritional value of ripe fruits that developed from treated flowers was equal to that of fully seeded untreated fruits grown under the same conditions.

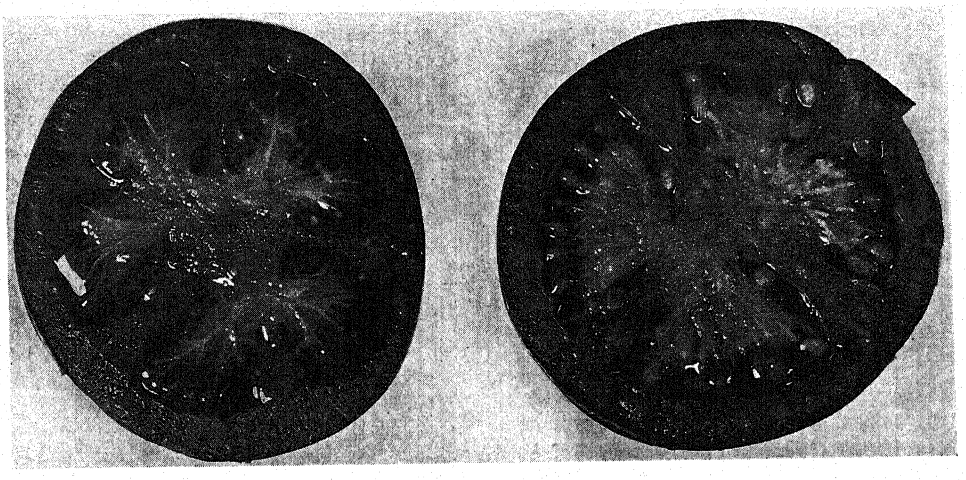


FIG. 2.—Ripe tomato fruits showing inhibited seed growth and amount of placental tissue and gelatinous pulp when previously treated with vaporized naphthoxyacetic acid (left) compared with untreated (right).

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LITERATURE CITED

1. DAVIS, W. B., Extraction of ascorbic acid from plant tissues. Ind. Eng. Chem. 34:217–218. 1942.
2. GUSTAFSON, F. G., Parthenocarpic and normal fruits compared as to percentage of setting and size. BOT. GAZ. 102:280–286. 1940.
3. ———, β-naphthoxyacetic acid as an inductor of parthenocarpy in tomatoes. Proc. Amer. Soc. Hort. Sci. 40:387–389. 1942.
4. HAMNER, K. C., LYON, C. B., and HAMNER, C. L., Effect of mineral nutrition on the ascorbic-acid content of the tomato. BOT. GAZ. 103:586–616. 1942.
5. HOWLETT, F. S., Use of chemicals to stimulate fruitfulness in tomatoes. Ann. Report Veg. Growers Assoc. of America 1941:1–13. 1941.
6. WONG, CHEONG-YIN, Progress report on induced parthenocarpy in some horticultural crops. Proc. Amer. Soc. Hort. Sci. 37:158–160. 1939.
7. ZIMMERMAN, P. W., Growth regulators of plants and formative effects induced with β-naphthoxy compounds. Proc. Nat. Acad. Sci. 27:381–388. 1941.

CURRENT LITERATURE

The Reproductive Capacity of Plants. By E. J. SALISBURY. London: Bell and Sons, 1942. Pp. xi+244. Figs. 35. 30/.

The author presents data, many of them analyzed statistically, gathered during the past 15 years on the reproductive capacity of a species and the possible ecological importance of such capacity. The abundance of material adds greatly to the relatively small amount of information previously available. Among many others, the following points are made by the author:

1. The average and potential progenies of a species are as much a specific characteristic as are its average and potential heights, although, like the latter, subject to a considerable range of normal variation.

2. The seed or fruit weight tends to augment with the degree of shade to which the seedling is normally subjected. In general, it would appear that the more advanced the phase of succession with which the species is naturally associated the greater the amount of reserve food material provided in the seed.

3. The capacity to colonize in the face of competition appears to be associated with the amount of food reserve which the seed contains.

4. It would generally appear to be true that, for ecologically comparable species, the magnitude of the reproductive capacity is associated with the frequency and abundance, of which it is probably one of the determining factors. On the other hand, the magnitude of the reproductive capacity appears to bear little if any relation to the risks of mortality, although a certain minimum is obviously necessary to meet those risks, but probably, for most species at least, the seed output is considerably in excess of that minimum.

5. The lowest seed outputs characterize the herbaceous shade species of woodlands, in which the increased pressure of competition and shading to which the germinating seedlings are subject is met by increased seed weight and/or vegetative means of propagation.

6. For most species at least, the seed output is considerably in excess of that requisite for mere replacement of losses by death and sufficiently so to bear no obvious relation to the normal seedling mortality.

7. Some of the most successful species are those which exhibit a large seed output and possess also a means of vegetative propagation, whilst species in which vegetative propagation is the only or dominant method of reproduction are mostly associated with the more densely populated habitats and the later phases of the plant succession.

8. A high reproductive capacity, in the views hitherto orthodox, might be rightly held to be in the nature of a mark of failure, whereas the evidence furnished has shown that a high potential rate of increase is, indeed, rather a criterion of success.—E. J. KRAUS.

Plasmodiophorales. By JOHN S. KARLING. New York: Published by the author, 1942. Pp. ix+144. Illustrated.

The publication of a monograph, beautifully illustrated and comparative, of the difficult group of the Plasmodiophorales will be welcomed by botanists and plant pathologists. This group includes eight recognized genera: *Plasmodiophora*, *Tetramyxa*, *Sorosphaera*, *Sorodiscus*, *Spongospora*, *Ligniera*, and *Polymyxa*. Of these, *Plasmodiophora* is the best known.

The small size of the organisms, the unusual cytological phenomena, and the relations of the group to other fungi and of the members of the group to one another make them very confusing to the average biologist, and this effort to bring together, compare, and evaluate the widely scattered observations is to be commended. Even the terms used in describing them are so unusual that a glossary precedes the general discussion.

In the chapters on the general cytology of the group and of the various genera and species, the author is on familiar ground. The small size of the nuclei, cells, and spindle figures, together with the poorly defined outlines of the cells in host plants, make accurate observations difficult and lead to theorizing on life histories, alternation of generations, and to the inclusion of doubtful species in the various genera. The position of these excluded or doubtful species has not been indicated, however, and the reader wonders what the author would do with them.

Plant pathologists will find in the two chapters on clubroot of cabbages and other cruciferous plants, and that on powdery scab of potatoes, a more detailed account of the parasites and the diseases which they cause than may be found in the larger plant disease manuals. The chapter on clubroot discusses resistant and susceptible varieties and methods of control, with data and observations compiled from all previous investigators. The chapter on the phylogeny and relationships of the group and of its members is outstanding.

The plates and text figures are accurately and compactly redrawn from various publications, and

the diagrams illustrating the various theories on life histories are excellent. Bibliographies are supplied for each chapter and are as complete as existing world conditions make possible. The book will be indispensable to the cytologist, botanist, and plant pathologist.—B. F. LUTMAN.

Ornamental American Shrubs. By WILLIAM R. VAN DERSAL. New York: Oxford University Press, 1942. Pp. xiii+288. Illustrated. $4.00.

The book points out the possibilities for the enrichment and improvement of American gardens through the extended use of many more native shrubs than is now the case. The author briefly indicates what those who have tried to cultivate native shrubs quickly learn by experience—that many of them are both disappointing and difficult subjects under cultivation. Some of the most useful are given special mention. It is also undoubtedly true that additional varieties and forms may be selected which have greater horticultural value, including under that designation their relative freedom from insect and disease attack and their responses to horticultural practices such as cultivation, pruning, etc.

A valuable part of the discussion concerns the plant-growth regions of the United States; more than thirty are listed. Shrubs which are considered desirable horticulturally are presented in groups, based on their botanical classification, and are then described in greater detail. The suggestions as to the regions in which they are likely to succeed should prove particularly valuable.

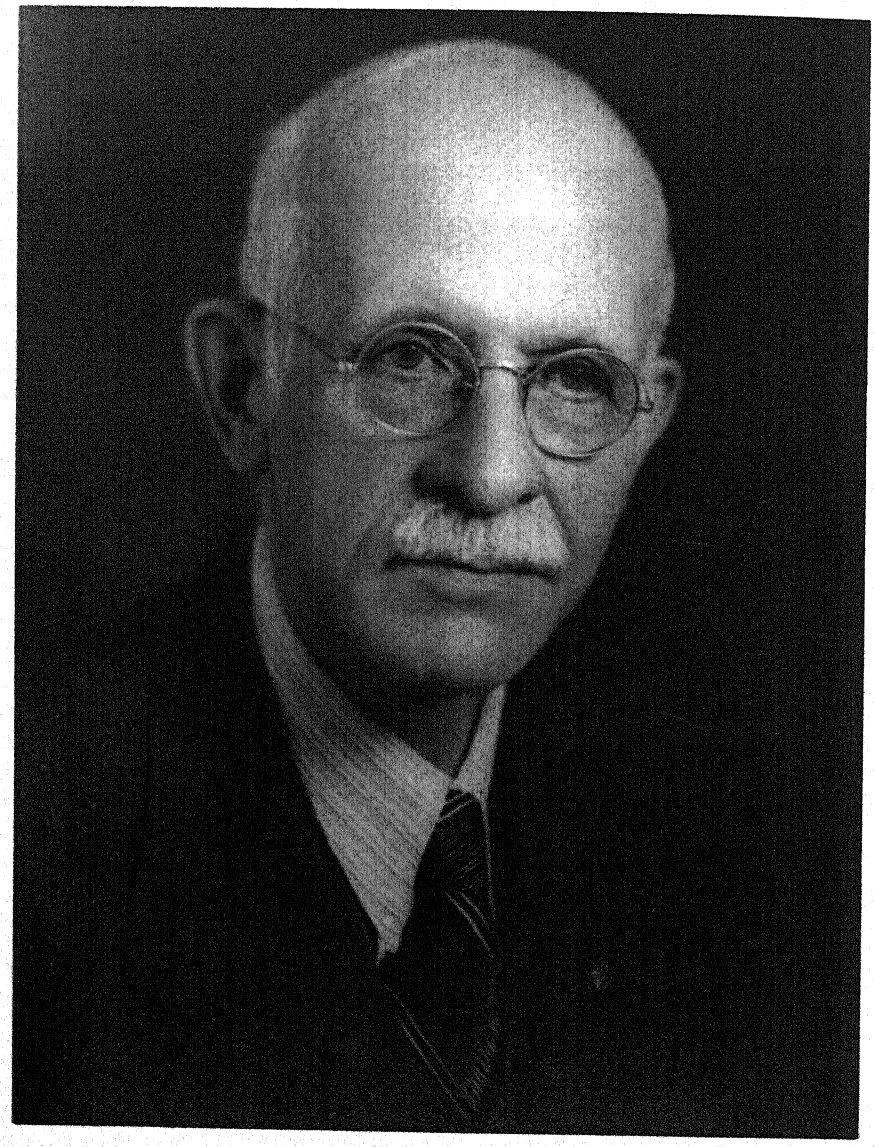
The descriptions are in the main clear, objective, and relatively free from the poetic subjectivity and effusiveness which so frequently confuse discussions on ornamental plants. The illustrations are liberal in number, purposeful, and of outstanding merit and beauty. In places a somewhat flippant style adds nothing of value to the text. Some of the discussion dealing with plant exploration under the general heading of looting seems neither appropriate nor needful, if one considers the valuable information obtained as a result of transplantation of American flora abroad, and in turn the nearly incalculable gains from the introduction of many foreign species into America. The work is thoroughly readable, however, and makes a definite contribution to horticultural literature.—E. J. KRAUS.

Weed Control. By WILFRED W. ROBBINS, ALDEN S. CRAFTS, and RICHARD N. RAYNOR. New York: McGraw-Hill Book Co., 1942. Pp. xi+543. Illustrated.

This volume goes far toward filling a great need for information on weeds and their control. It is comprehensive in scope, both in type of subject matter treated and in the detail with which it is presented. Principles and methods of control, critical evaluation of some chemical treatments, and the handling of special problems are presented with sufficient consideration and detail to provide a clear and definite background for dealing with the weed problem. There is also a list of some weeds frequently encountered and regarded as of special economic importance. Data are presented by means of clear tables, graphs, and many excellent, fresh, meaningful illustrations. Running comments, backed by the experiences, contacts, and critical judgments of the authors combine to make a reference and guide of more than usual value.—E. J. KRAUS.

Forest Tree Seed. By HENRY IVES BALDWIN. Waltham, Massachusetts: Chronica Botanica Co., 1942. Pp. xvi+240. Figs. 28. $4.75.

The book deals comprehensively with the structure, development and origin, collection, cleaning and storage, germination, biotic enemies, and certification of tree seeds. There is also a chapter suggesting additional research problems. The material presented is critically examined, well documented, and there are fairly extensive lists of references to literature. Much of the discussion on harvesting, storage, and germination pertains with equal validity to many types of plants, so that the book has a wide field of interest and usefulness beyond the more immediate limits of species of trees.—E. J. KRAUS.



CHARLES JOSEPH CHAMBERLAIN

(WITH PORTRAIT)

The death of Professor CHAMBERLAIN on January 5, 1943, marks the passing of the last of those who, in the nineties, pioneered in establishing the Department of Botany at the University of Chicago. Dr. CHAMBERLAIN was a graduate student on the campus before any botanical courses were offered, and his insistent desire for such work was no small factor in bringing about the establishment of botanical courses and the early organization of the department. He was one of the group on hand to welcome Dr. JOHN M. COULTER in 1894, when he first made weekly visits to the campus for lectures and conferences. As Assistant and Associate, Dr. CHAMBERLAIN was in charge of the botanical laboratories from the beginning. He was the first to receive the Doctor's degree from the department (in 1897), was Instructor in 1901, Assistant Professor in 1907, Associate Professor in 1911, and Professor of Morphology and Cytology from 1915 until his retirement as Professor Emeritus in 1929. After his retirement he remained active, lecturing at various institutions and continuing his writing and his research—especially on the morphology and systematics of the cycads.

CHARLES JOSEPH CHAMBERLAIN was born near Sullivan, Ohio, on February 23, 1863. He received the usual rural school education, and attended high school in Oberlin, completing the three-year course in two years. This was followed by a year in the preparatory department and four years in Oberlin College, where he completed the classical course for the A.B. degree in 1888. He taught in the public schools for five years after graduation, including the Crookston (Minn.) high school, where he was Principal, 1889–1893.

His early attraction to botany came from interest inspired by Prof. A. A. WRIGHT of Oberlin College, a geologist who taught botany and zoölogy with equal enthusiasm. Returning to Oberlin during the summers, Dr. CHAMBERLAIN completed the requirements there in botany for the M.A. degree (1894). He was awarded the D.Sc. from the same Institution in 1923. At Oberlin he first saw the celloidin method and a sliding microtome used on animal tissues. During his first year at Chicago, zoölogical courses acquainted him with the newer techniques in animal histology and cytology. This training constituted his foundation for the adaptation of these and other methods in the study of plant tissues. His volume, Methods in Plant Histology, still the standard book of its kind, first appeared in 1901, and was enlarged and revised through five editions to 1932.

Close collaboration with Professor COULTER in teaching and research resulted in the publication of the Morphology of Seed Plants (1901), the Morphology of Angiosperms (1903), and the Morphology of Gymnosperms (1910, 1917). His books also include The Living Cycads (1919), Elements of Plant Science (1930; translated into Spanish, Buenos Aires, 1942), and Gymnosperms: Structure and Evolution (1935). His monograph on the cycads had not reached publication stage at the time of his passing, but it is so

nearly completed that it may be expected to appear soon.

His botanical researches are too numerous to mention in detail and include investigations in all major groups of plants as well as gymnosperms. The investigations of cycads at Chicago were inspired by him and the material he provided. His special interest grew out of a collaborative investigation with Dr. COULTER on the embryogeny of *Zamia*, which led to the realization of the general lack of knowledge concerning the other cycad genera, mostly tropical. While in Germany at Bonn (1901–02) for a year's research in Professor STRASBURGER'S laboratory, he learned the approximate location of *Dioon edule* in Mexico. After his return from Europe he made a collecting trip into Mexico, which resulted in the discovery of several excellent cycad localities. Four trips to that country, together with an arrangement for the periodic shipment of cones and plant material to Chicago, provided him with material of *Dioon edule*, *D. spinulosum*, and *Ceratozamia mexicana*. He visited Cuba in 1914, and again in 1922, in the study of *Microcycas*. His most extensive excursion for cycads was made in 1911–12 to New Zealand and Australia, returning via South Africa, for a study in their habitats of the remaining genera and most of the species.

These collections resulted in the assembling in the botanical greenhouses at the University of Chicago of the most extensive and complete collection of cycads ever brought together. His book, The Living Cycads, includes an account of these botanical excursions. This initial interest in cycads, and the stimulation of much research in this field, have developed the knowledge of the group, so that from the least known they are now the best known of the gymnosperms.

Professor CHAMBERLAIN was well known internationally as a foreign or corresponding member of botanical societies in England, Germany, Switzerland, India, and elsewhere. In 1923 he was Vice President and Chairman of the Botanical Section of the American Association for the Advancement of Science and was elected President of the Botanical Society of America in 1931. Among other activities, he was for many years American editor for cytology in Botanisches Zentralblatt; he was also Associate Editor of the Botanical Gazette.

As a boy, early musical talent had led his parents to expect that he might pursue music as a profession, but he contented himself with participation in orchestras, bands, and choral organizations of the college and community, a type of activity which remained an avocation throughout life. In Chicago he served for 10 years as director of music at the Woodlawn Baptist Church and was a member of the Apollo Club for 35 years. When a youth, he had learned to transcribe and arrange band music, and some of his original compositions were played by the town band. One of his musical themes was rendered on the Chapel organ at his funeral service.

During World War I, he joined in activities of the Rifle Club organized by Dr. W. J. G. LAND and soon qualified as Sharpshooter at Fort Sheridan, later as Expert Rifleman in both the Navy and unified Army-Navy courses.

Not only his family, but his wide circle of friends and colleagues, will miss him. His former students, now found in all parts of the world, gratefully remember his kindly but critical advice and counsel and will always cherish his memory as a friend. His passing is a deep personal loss to all of us.—J. T. BUCHHOLZ, *University of Illinois, Urbana, Illinois.*

VEGETATION AND SUBSTRATE OF CRANBERRY GLADES, WEST VIRGINIA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 551

H. CLAYTON DARLINGTON

(WITH SEVENTEEN FIGURES)

Introduction

In several parts of West Virginia there are small isolated areas of bog or peatland ranging in elevation from 2500 to 3500 feet. The most important in size and peculiar vegetation are the Cranberry Glades in Pocahontas County. These occur in a level area of about 600 acres, at an elevation of 3375 feet, surrounded by hills ranging in elevation from 4000 to 4600 feet. Approximately 400 acres are covered with shrubs; about 80 acres with bog forest; and about 120 acres with sedges, mosses, and lichens. The latter 120 acres are popularly called "glade," four divisions of which are designated as Big Glade (approximately 60 acres), Flag Glade (28 acres), Long Glade (20 acres), and Round Glade (8 acres). Six different plant communities were recognized at the beginning of the study as worthy of investigation: bog forest, sedge-sphagnum, moss-lichen, sphagnum-cranberry-beaked rush, and two shrub communities.

The area lies in the southwestern part of Pocahontas County, about 20 miles west of Allegheny Mountain and the Virginia state line, and 9 miles west of Marlinton, the county seat of Pocahontas County (fig. 1). It is at the headwaters of Cranberry River, which flows through the bog. The area may be reached at present by turning off from Federal Route 219 at Millpoint and following the road up Stamping Creek to its head on Cranberry Mountain, the round trip totaling 12–15 miles. One then must walk 7 miles on a round trip from the top of Cranberry Mountain to the different parts of the bog.

The objects of the present investigation were: (*a*) to examine the geology of the region and the profile of the bog sediments with the hope of finding some explanation for the formation of the level bog area; (*b*) to examine the layers and the pollen of the peat to shed some light on the past history of the vegetation of the region; (*c*) to study the various ecological factors and their influence in producing the present forest, shrub, sedge, and moss communities; and (*d*) to observe successional trends and try to determine the probable future of the area. The study has extended over a period of 12 years. Previous to 1941 only two or

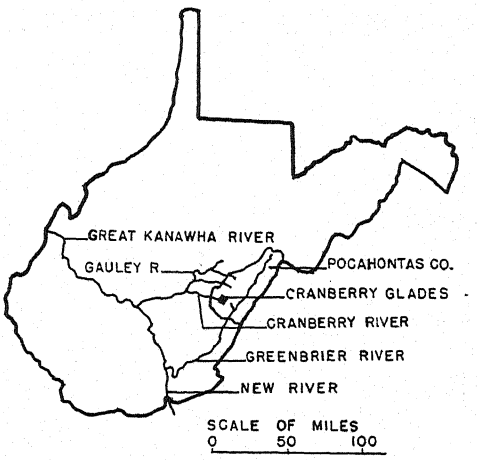


FIG. 1.—Location of Cranberry Glades, West Virginia.

three trips were made to the bog each year, but beginning with April, 1941, full time was devoted to the work until November of that year.

BROOKS (1, 2) listed some of the common plants, birds, and mammals of Cranberry Glades and estimated the approximate acreage of the different glades. He noted the importance of the area on water flow. SHELDON (11) collected and identified many species of fungi from this region. STRAUSBAUGH (12) discussed some of the bog species and also some of the rare plants of the area, such as *Cornus canadensis*.[1]

Climate

General climatic conditions in Pocahontas County can be divided into two classes, the highland climate and the low valley climate. The valleys have an elevation of 2100–2400 feet, while the highlands reach 3000–4600 feet. Winters are severe in both but are more severe in the highlands. Summers are cool, with the maximum day temperature in July rarely above 88° F. in the valleys or 80° F. in the highlands. Night temperatures may drop as low as 55° F. in Cranberry Glades in July.

While there is no complete record of climatic factors in the highlands, data have been kept for 30 years by S. L. BROWN at Marlinton (2125 feet). Temperatures throughout this paper are given in Fahrenheit and precipitation in inches. These records show a 30-year mean air temperature of 48°, with means of 67.6° and 27.7° for July and January. The maximum mean for July was 71.2° in 1898; the minimum mean, 62.3° in 1920. The highest mean for January was 34.4° in 1913; the lowest was 16° in 1918. There is no record available for the absolute maximum and minimum during this 30-year period; however, in Cranberry Glades during the summer of 1941 the maximum recorded was 82° on July 22.

The 30-year mean in precipitation is 47.28 inches. July has the highest monthly mean (5.31 inches); November the lowest (2.93 inches). The highest annual rainfall recorded was 63.62 in 1918, and the lowest was 39.54 in 1912. The rainfall is fairly evenly distributed through the months of the year, September, October, and November having the lowest monthly values and June, July, and August the highest. This is favorable to plant growth. In snowfall the 30-year mean is 31.5 inches, with 9.5 for January, 9.2 for February, 5.8 for March, 1.2 for April, a trace for May, 0.6 for October, 1.6 for November, and 6.3 for December. No snow has been reported for June, July, August, and September. The maximum annual fall was 48.5 inches for 1916; the minimum annual was 11.5 for 1913.

Mean length of the frost-free period for the 30-year period is 146 days, May 15 to October 8. The maximum was in 1919, April 19 to October 20 (183 days), while the minimum was in 1913, June 10 to September 23 (104 days). For the Cranberry Glades region it would be a close approximation to put the mean annual temperature at 42°, the mean annual rainfall at 51 inches, the mean annual snowfall at 40 inches, and the mean frost-free period at 140 days. Although not present to record the temperature, the writer has satisfactory evidence in the appearance of the plants that in 1941 there was frost on May 15 and October 2, a frost-free period of 140 days.

Physiography and geology

The area is in the Allegheny Plateau subdivision of the Appalachian Province.

[1] With the exception of lichens and mosses, the nomenclature used is that of GRAY'S New manual of botany, 7th ed.

The rocks exposed are sandstones and shales, which, with the limestone beneath, were deposited in a vast inland sea during Mississippian and Pennsylvanian time and were uplifted at the close of the Permian (table 1). The region of greatest uplift was farther east, causing all strata in this area to dip to the west.

TABLE 1

GENERAL COLUMNAR SECTION OF ROCKS EXPOSED IN CRANBERRY GLADES AREA OR LYING BENEATH IT

Era	Period	Series	Group	Thickness (feet)	Description
Quaternary	Recent			?	Unconsolidated clays and gravel
	Pleistocene			?	Unconsolidated clays and gravel
Paleozoic	Upper Carboniferous or Pennsylvanian	Pottsville	Kanawha (part)	130	Sandstones, shales, coals
			New River	500	Massive sandstones, hard dark shales, coals
		Mauch Chunk	Bluestone	75–200	Vari-colored soft shales and sandstones
			Princeton conglomerate	50– 70	Massive gray and brown sandstones with pebbles
			Hinton	500–700	White weathering sandstones, variegated shales (soft)
	Lower Carboniferous or Mississippian		Bluefield	550–800	Variegated shales, sandstones, and limestone
		Greenbrier		220–600	Limestone of various colors (gray, blue, light gray)
		Maccrady		20– 50	Red and purple shales
		Pocono		60–600	Sandstones and shales

During Cretaceous or Jura-Cretaceous time the Allegheny Plateau was eroded by water action to an almost perfect base level, the Schooley peneplain. Another uplift at the close of the Cretaceous was followed by peneplanation in the less resistant areas to the Harrisburg peneplain in Tertiary time. Black Mountain, with an elevation of 4600 feet, and other hills around the bog area are remnants of the Schooley peneplain. Still another uplift occurred at the close of the Tertiary, resulting in further dissection and erosion.

The level bog area, apparently a part of the Harrisburg peneplain, is 3 miles long and $\frac{1}{4}$–1 mile wide. It lies at an elevation of a little less than 3400 feet at the upper or eastern end and 3350 feet at the lower end (fig. 2). It is apparently level but has a drop of 45 feet in 3 miles.

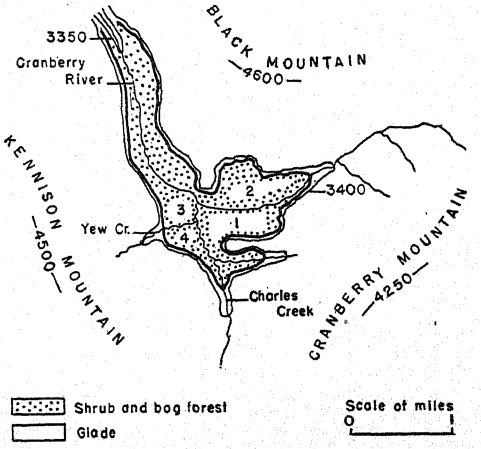


FIG. 2.—Level bog area. Dotted area shows location of shrub and bog forest of about 480 acres; white area represents open glade. Nos. 1, 2, 3, and 4 show, respectively, location of Big Glade (60 acres), Long Glade (20 acres), Flag Glade (28 acres), and Round Glade (8 acres).

Cranberry River has its source in springs on Cranberry Mountain $1\frac{1}{2}$ miles east of the bog. This stream, about 12 feet in width, flows through the bog and is joined in the lower part by Charles Creek, formed from small streams from both Cranberry and Kennison mountains. These streams meander extensively through the bog, with the exception of the new and almost straight course of Cranberry River along the north side of Big Glade—which has cut off some of its largest meanders (fig. 3). Feeding these streams are hundreds of cold springs on the hillsides, and especially on Cranberry Mountain, which has many more springs than Black or Kennison mountains, owing to a difference in geological formation. Cranberry Mountain is composed entirely of Mauch Chunk shales and sandstones, which in erosion produce wide flat-topped hills very receptive to rainfall and water from melting snows, thus allowing continual spring and seep-

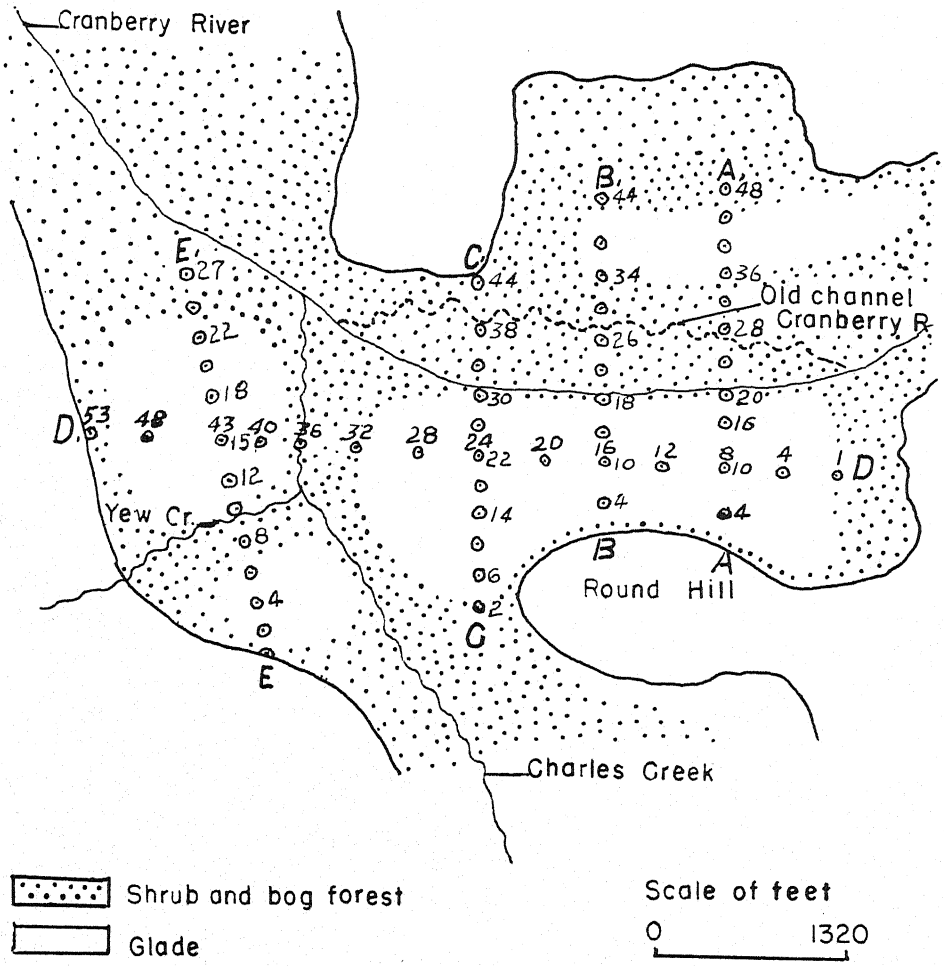


FIG. 3.—Apparently level bog area with location of transect profiles. Transect: A-A_1, with 48 borings at 50-foot intervals; B-B_1, with 44 borings at 50-foot intervals; C-C_1, with 44 borings at 50-foot intervals; D-D_1, with 53 borings at 100-foot intervals; E-E_1, with 27 borings at 100-foot intervals.

age flow from the hillsides. Black and Kennison mountains are capped by 500 feet of New River sandstone and massive shales of the Pottsville series, which in base level, but streams $1\frac{1}{2}$ miles from their spring source and 3400 feet above sea level usually cut V-shaped valleys, as Cranberry River is doing below the bogs.

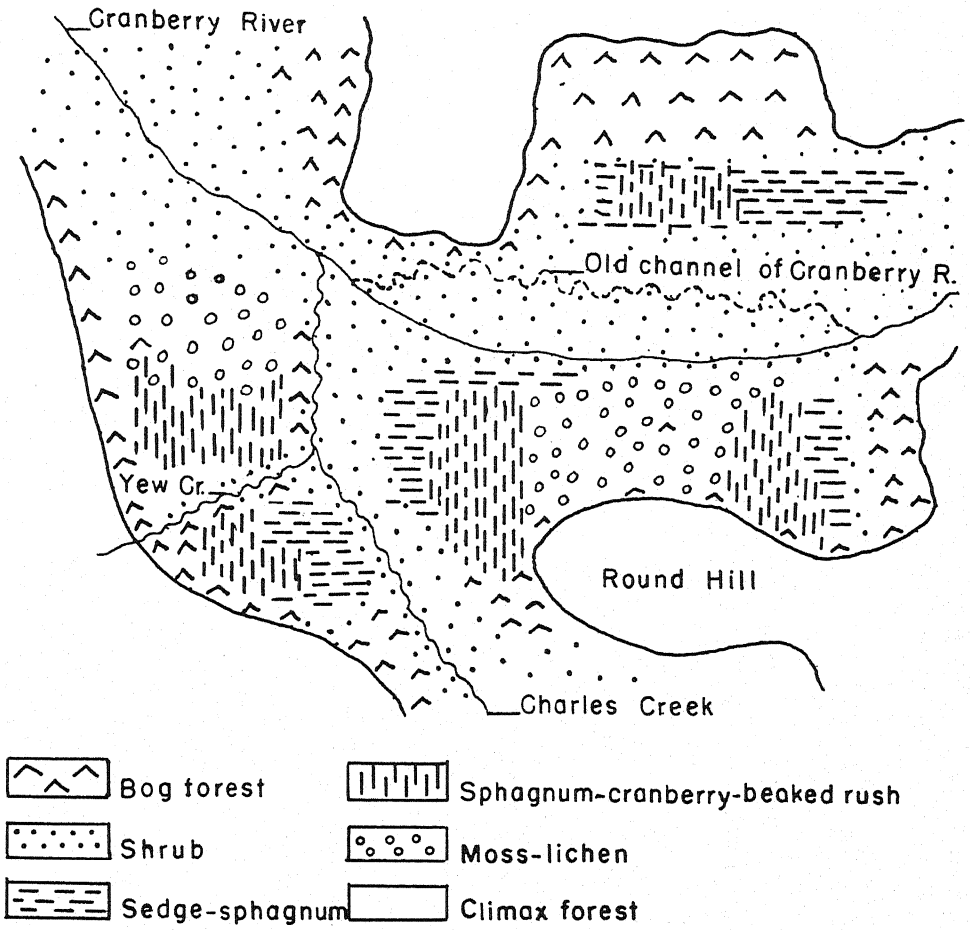


FIG. 4.—Community distribution over the area

erosion form sharp ridges not receptive to rainfall and melting snow.

ORIGIN OF LEVEL AREA

The writer early was confronted with the puzzling question of the origin of a wide valley so near the source of a stream. This is no ordinary occurrence. Mature rivers may develop wide valleys by lateral cutting when approaching So far as can be learned, no explanation of this unusual situation has been attempted other than a suggestion that a mountain lake has been filled with sediments and plant remains, thus producing the level area. Yet before a lake could have existed, the widened valley had to be formed. Two possible methods of widening were given consideration.

1. ORIGIN BY SINKING.—Solution of

limestone below shales and sandstones may cause a collapse of strata, thus forming depressions and often lakes. The fact that the bottom of the peat at the center of Big Glade (figs. 8, 9) is 8 feet lower than the bank of Charles Creek at the lower end of this glade is suggestive of a sink here. Furthermore, the Greenbrier limestone lies beneath this area at an elevation sufficiently above the valley of Hill's Creek to the south to permit underground drainage of the Cranberry Glades area to the Greenbrier River through Hill's and Locust creeks. However, the strata beneath the bog consist of at least 800 feet of shales and sandstones, which should stand up—even with solution of the limestones beneath. It is therefore improbable that there has been any collapse of strata here to form or to aid in the formation of the widened valley by erosion.

2. ORIGIN BY SURFACE EROSION.—With the uplift of the Schooley peneplain at the close of the Cretaceous, the streams began anew their work of erosion and dissection. Greenbrier River took its course southwestward with the outcrop of the less-resistant strata of the Mauch Chunk and Greenbrier series. Cranberry River and others to the north took their courses to the northwestward with the slope of the peneplain, due to warping in the uplift. The location of the head of Cranberry River is in a critical position in two respects: (*a*) it is near the beginning of the slope of the peneplain westward; (*b*) it is in a location of increased dip of strata. Measurements show that from the top of Cranberry Mountain to the center of the bog area, a distance of 3 miles, the dip is 275 feet to the mile, while for the next 5 miles downstream the dip is 125 feet to the mile. This condition caused the upper few miles of the stream to cut in mostly softer rocks, while the remainder was cutting in hard sandstones and conglomerates.

During Tertiary time Greenbrier River cut down much faster than Cranberry River because it was cutting in soft rock while the latter was cutting in very resistant sandstones and conglomerates. By the close of the Tertiary, Greenbrier River had cut down to near the bottom of the Greenbrier limestone, 2100 feet below the Schooley level, and had formed a partial peneplain (Harrisburg) in the less resistant area—as evidenced by the Hillsbora plain, Jerico Flats, Key Flats, and other points. During the same time, Cranberry River and its branch, Charles Creek, had cut down about 1200 feet. The upper parts, cutting in soft strata, reached temporary base level in the Mauch Chunk shales, the level being controlled by the hard layers downstream. Sideward cutting in soft rock then allowed the streams to widen their valley to these unusual widths. It is to be noted, however, that they were considerably longer then than now and were gradually shortened by the rapid downward cutting of Greenbrier River and the piracy of its branch, Stamping Creek, westward into the drainage basin of Cranberry River and its branch, Charles Creek, thus moving the divide farther westward. The widened valley is thus a part of the Harrisburg peneplain, the writer believes, although it is 1000 feet above the Hillsbora level.

Another uplift at the close of the Tertiary caused the Greenbrier to entrench 400 feet into the Harrisburg peneplain to the present level. No such entrenching occurred in the Cranberry Glades area, owing to the slow cutting of the river in the very resistant conglomerate below the level area. Widening probably continued, but more slowly, as the rejuvenation of the branches of Greenbrier River

led to further piracy and shortening of Cranberry River and Charles Creek.

Soils

Soil of surrounding hills

The profiles of the bog were examined carefully, and some attention also was given to the soil of the surrounding area to the top of Kennison Mountain, 1100 feet above the bog. The soils of Cranberry Mountain and the lower parts of Kennison and Black mountains are derived from mixtures of the many-colored shales and sandstones of the Mauch Chunk series with more or less humus. On Cranberry Mountain the soil is deeper and contains more humus than on Black Mountain, because of less exposure to the sun, more spring and seepage flow, and fewer fires. Kennison Mountain is intermediate in these respects. Soil tests for acidity in the surrounding area show a range from pH 4 in spruce forest on top of Kennison Mountain to pH 6.2 on Cranberry Mountain in maple-birch forest. No neutral or alkaline soil was found.

Soil of bog

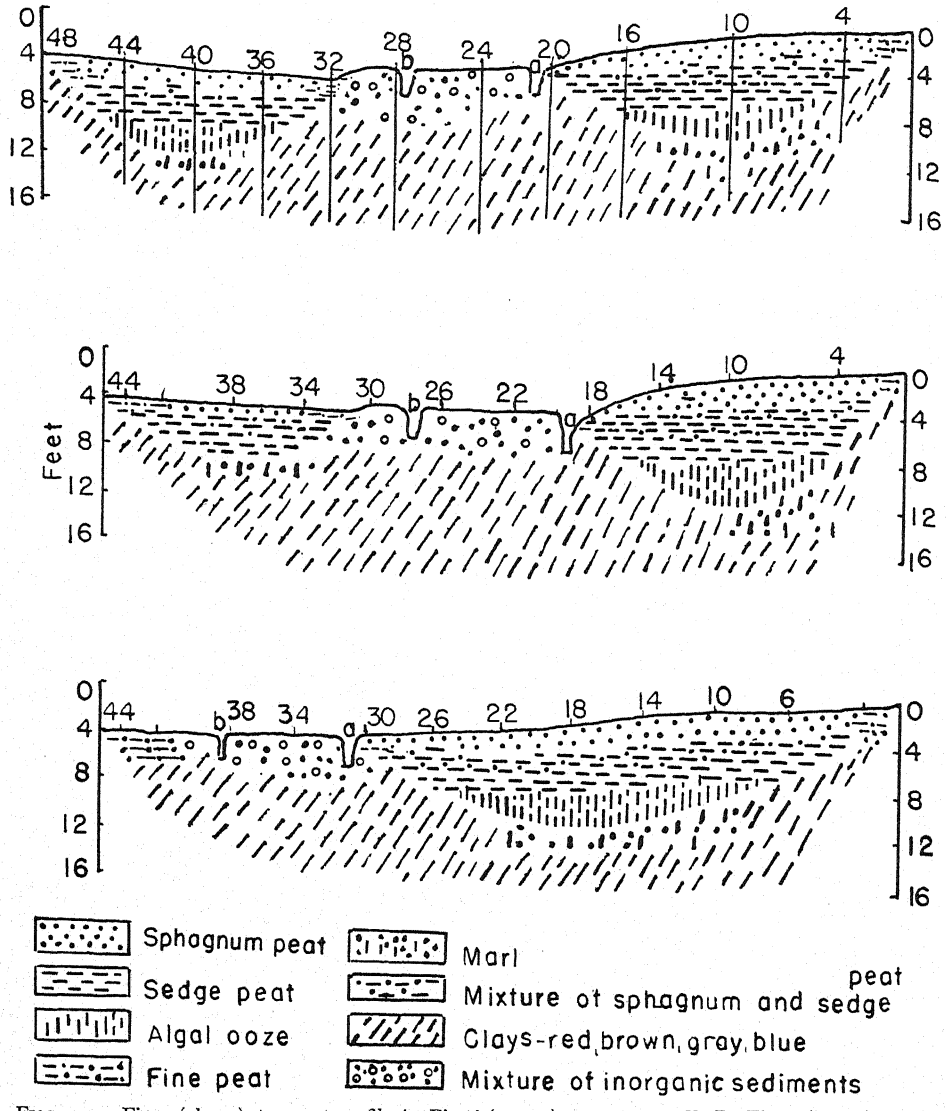
Figure 3 shows the location of many profile samples along various transects in the area. All parts of the bog have been sampled in order to obtain a clear picture of the accumulated sediments and peat. More than 150 borings were made, with a Hillier peat-borer.

Transect A.—This transect passes from Round Hill on the south directly north across the middle of the upper half of Big Glade, across a belt of alders along Cranberry River, and across Long Glade into spruce forest north of Long Glade, a distance of 2400 feet (fig. 3). Borings were made at intervals of 50 feet to varying depths. Generally samples were taken as deeply as the borer could be pushed into the very stiff compact clay. Fifteen feet was the maximum depth from which it could be recovered by two men.

The profile revealed by this transect is shown in figure 5. Stations 1–20 show the profile of Big Glade, beginning at its southern margin at Round Hill. Station 1 is 50 feet inside the margin of the bog and shows 4 feet of fine peat with many logs. Below this are 2 feet of clay and sand underlain by rock. Between this station and the base of the hill several other borings showed that the peat thinned to 1 foot at a distance of 3 feet from the base of the hill and was only 1 foot above rocky material. Station 2, 100 feet from the base of the hill, shows 5 feet of fine peat overlying 3 feet of a mixture of clay and organic material underlain by very compact clay. Stations 1 and 2 are within a belt of shrubs, about 125 feet wide. Station 3 is 25 feet from the border of the shrub belt, in the glade. Samples taken here show $6\frac{1}{2}$ feet of peat, including $1\frac{1}{2}$ feet of sphagnum peat at the top, 3 feet of sedge peat mixed with sphagnum peat, 1 foot of sedge peat, and 1 foot of algal ooze. Below the peat are 2 feet of compact sticky clay. The next eight borings show similar profiles, with a gradual thickening of the peat to $8\frac{1}{2}$ feet at the midway point across Big Glade. Station 11, 15 feet in depth, shows respectively from top downward 2 feet of sphagnum peat, $2\frac{1}{2}$ feet of sphagnum and sedge peat mixed, 2 feet of sedge peat, 2 feet of algal ooze, 3 feet of marl, and $3\frac{1}{2}$ feet of clay. The next nine stations show a similar profile, with gradual thinning of the peat to 2 feet at station 20 at the border of the shrub belt on Cranberry River. Underneath the peat is clay as far as samples could be taken. Station 21 is on the north side of Cranberry River,

and a boring made with a 2-inch pipe to a depth of 11 feet shows only fine sediments of clay and sand.

5 feet deep. It may overflow its banks during floods and deposit inorganic sediments. As a result, stations 21–27 show



FIGS. 5–7.—Fig. 5 (above), transect profile A. Fig. 6 (center), transect profile B. Fig. 7 (below), transect profile C. Horizontally, 1 inch equals 530 feet; vertically, 1 inch equals 16 feet. Vertical exaggeration is approximately 33. *a*, present location of Cranberry River; *b*, old channel.

From Big Glade, transect A crosses Cranberry River and a belt of shrubs, mostly alders, 660 feet wide between Big Glade and Long Glade. Cranberry River is about 12 feet wide here and in flood is

a profile of fine sediments, mixed with organic materials in some places. They were very difficult to bore. Stations 29–34 show peat 2–5½ feet in thickness, with clay beneath, as in stations 1 and 2.

Stations 35–44 are in Long Glade, with a profile similar to those in Big Glade, although the peat is only 7 feet thick at the deepest part. Stations 45–48 extend through a belt of alders on the north side of Long Glade and into spruce forest. Here the peat is 4 feet thick at station 44 and thins to 1 foot at station 48. Underneath the peat are 3–4 feet of red clay from the red Mauch Chunk shales on the hills to the north.

TRANSECT B.—This transect, beginning at Round Hill on the south side of Big Glade (figs. 3, 6), crosses an alder belt 120 feet in width, passes through the center of Big Glade, and then crosses a shrub belt to slightly north of the western end of Long Glade. The total length is 2200 feet. Stations 1–3 are similar to 1–3 in transect A. Stations 4–18 are similar to 4–20 in transect A, except that the peat is 2 feet thicker on the average and the thickness of the algal ooze is greater. Stations 10 and 11, near the center of Big Glade, show the deepest peat in any part of the entire area, 11 feet in thickness. Clay and sand extend below the peat 5 feet or more. At station 10 samples were taken for pollen analysis (figs. 3, 6, 13). Temperature readings (fig. 12) were taken here also. The different layers represented are: 2 feet of sphagnum peat, 3 feet of sedge and sphagnum peat mixed, 2 feet of sedge peat, 4 feet of algal ooze, 3 feet of marl, and 1 foot or more of clay.

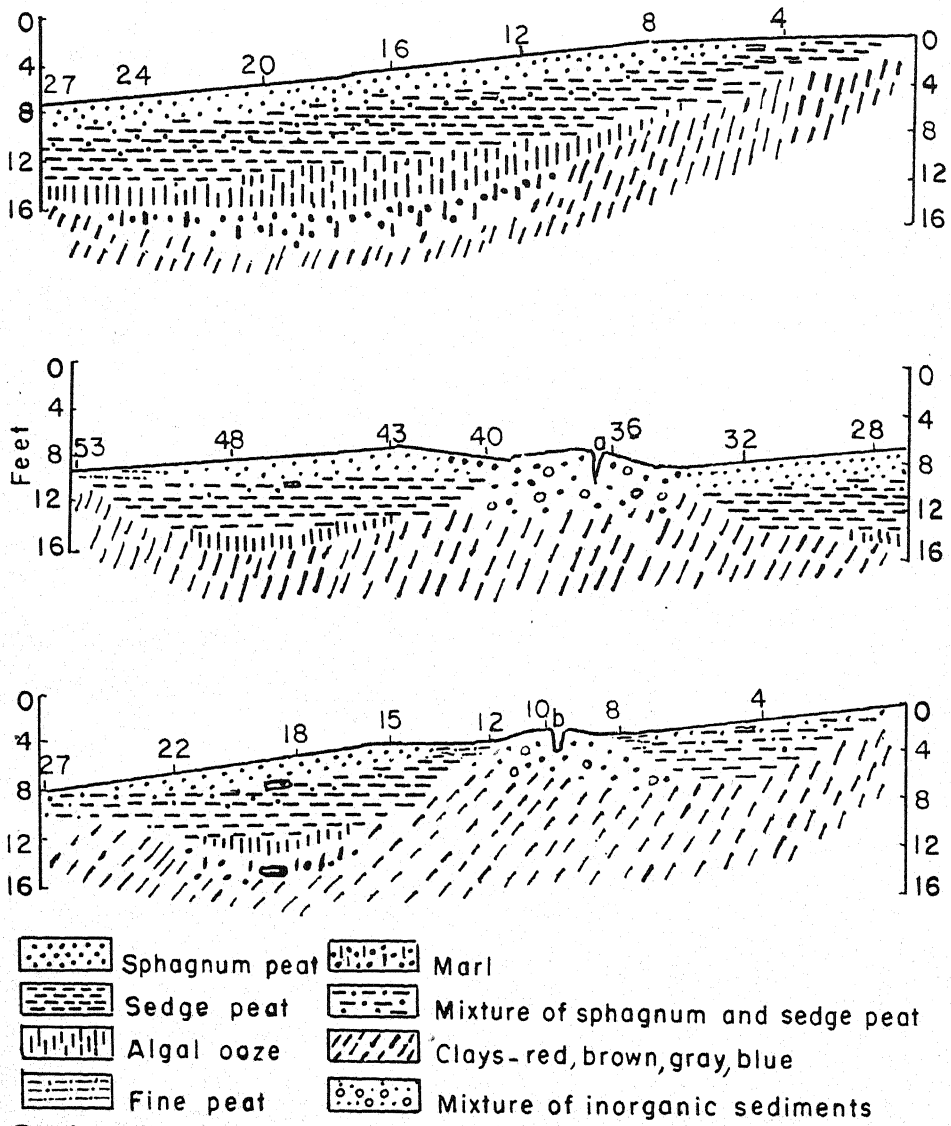
This area, showing the greatest depth of sediments, appears to be a sink in the general surface underlying the peat and supports the theory of formation of the level area by solution of limestone beneath shales and sandstones. Since it is unlikely that there has been any sinking, however, the depression will be explained by another process in a later section.

TRANSECT C.—This cuts across Big Glade in its lower part (figs. 3, 7). There are forty-four stations 50 feet apart, a total length of 2150 feet. It extends from the alder belt on the south side of Big Glade to the foot of Black Mountain. The section across Big Glade is similar to the corresponding section of transect A, with the exception of a 6-inch greater average thickness of peat. Stations 31–38 extend across the floodplain between Cranberry River and its old channel and reveal a profile of inorganic sediments similar to stations 21–28 of transect A. This area is practically level. Stations 39–44, which are north of the old channel, exhibit 1–1½ feet of fine peat accumulation. Station 30 of this transect, as well as 18 and 20 of transects B and A, respectively, shows that Cranberry River is now eroding into the peat of Big Glade.

TRANSECT D.—This is the longest transect, extending from the upper end of Big Glade in almost an east-west direction the full length of Big Glade, the belt of alders on Charles Creek, the width of Flag Glade, and the belt of alders and spruce west of Flag Glade to the foot of Kennison Mountain, a total length of 5200 feet (figs. 3, 8, 9). There are fifty-three stations at 100-foot intervals. It crosses transects A, B, and C at their deepest parts, and its profile is similar to theirs, with a gradual thinning of the peat toward either end of Big Glade but with almost a uniform thickness from transects A to C. The profile of the shrub belt on Charles Creek is similar to that of the other alder zones. In Flag Glade the peat is thinner than in Big Glade, not more than 8 feet thick at any point. The top surface of this profile shows a drop in elevation of 8 feet in the length of Big Glade, about equal to the drop in Cranberry River, which parallels it. There is a rise in elevation of 1 foot from the lower end of Big Glade to the bank of

Charles Creek. The lowest depth of peat in station 20 of this transect is 8 feet lower than the banks of Charles Creek at the lower or west end of Big Glade and 4 feet lower than the south bank of Cranberry River on the north side of Big Glade.

TRANSECT E.—This transect (figs. 3, 10) extends from the south side of Round Glade in a slightly northwest direction across Round Glade, the shrub belt on Yew Creek, Flag Glade, and into the alder zone north of Flag Glade. It includes twenty-seven stations at 100-foot inter-



FIGS. 8–10.—Fig. 8 (above) and fig. 9 (center), transect profile D. Fig. 10 (below), transect profile E. Horizontally, 1 inch equals 584 feet; vertically, 1 inch equals 16 feet. Vertical exaggeration is approximately 36.5. *a*, location of Charles Creek; *b*, of Yew Creek.

vals, totaling 2600 feet. Stations 2–7 show the profile of Round Glade, with the thinnest accumulation of peat of any of the glades, not exceeding 4½ feet. The peat is mostly sedge peat. Near the banks of Yew Creek the profile is similar to those cutting through the other streams, chiefly of inorganic sediments. The profile in Flag Glade is similar to that of Big Glade, but the peat does not exceed 8 feet at any point. The surface of the peat in Flag Glade shows a drop of 5 feet in elevation from the upper end at station 11 near Yew Creek to station 27 at the lower end.

WATER TABLE.—At the intersection of transects B and D in the center of Big Glade (fig. 3), a pit 5 feet deep was dug in 1934. The water level was read on a stake with an inch scale, driven into the pit, from April 12 to November 11, 1941 (fig. 11). On April 12 the water stood 1 inch below the peat surface, the highest recording at this station. On October 25 it was 12 inches below the surface, the lowest level recorded. This pit (designated as *A*) was in a *Polytrichum-Cladonia* community. A similar pit (*B*) was dug in Long Glade at station 38, transect A, in a *Sphagnum-Vaccinium-Rynchospora* community, and readings were made during the same period. Here the water level was 2 inches above the peat surface on April 12, and 7½ inches below on October 25. The water table was 2–5 inches higher continually than in pit *A*. Observations were made on the water table in the shrub belts along Cranberry River and Charles Creek. Pit *C* was located at station 31 of transect A in an *Alnus-Viburnum* community, and the shifting level was very similar to that of pit *B*. The lowest water table is between Cranberry River and its old channel on transect A, with a fluctuation of from 8 to 30 inches below the surface.

During 1935, 1937, and 1938, observations were made on the water table at irregular intervals. It is generally lowest in September and October.

SOIL TEMPERATURE.—At station 10, transect B, temperatures of the bog were taken from April 12 to November 11: at the surface of the peat beneath the moss, at the 6-inch depth, at the 1-foot depth, and at intervals of 1 foot down to a depth of 10 feet (1 foot above the bottom of the peat). A soil thermometer was used which was fastened to a pole for the lower levels. It was pushed into the peat to the required depth and left for 12–15 minutes, then pulled up quickly and the temperature recorded. Readings were taken between 2:00 P.M. and 4:30 P.M.

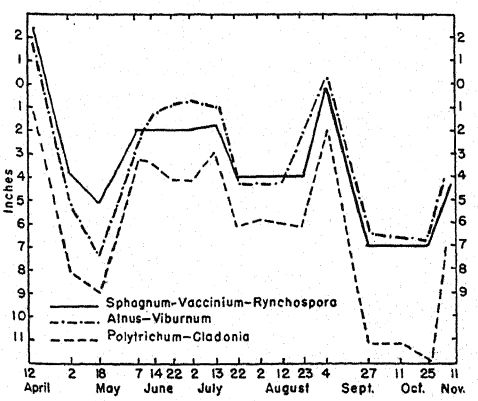


FIG. 11.—Fluctuations of water table in three communities; zero mark represents surface of peat underneath growing moss.

The surface temperature varied from 32° in April to 68° in August, while in the lowest level it rose gradually from 46° in April to 49½° in October (fig. 12). The low levels are little affected by the increase of heat on the surface through the summer. They reach their maximum temperature more than a month after the surface has reached its maximum and has begun to decline rapidly. Early in October these lower layers are still gaining in

heat and do not begin to show a net loss until the middle of October. Values for the 5–9 foot levels are omitted in figure 12. The 8- and 9-foot levels did not vary from the 10-foot level more than 1° at any time. The 5-, 6-, and 7-foot levels were 3°–6° colder in winter and 2°–5° warmer in summer than the 10-foot level.

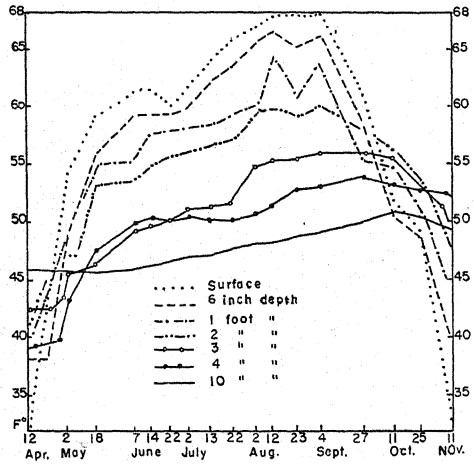


FIG. 12.—Temperature of bog profile at station 10 of transect B.

The temperature of the bog soil is no doubt influenced by the water from hundreds of cold springs which flows into it, especially from Cranberry Mountain (fig. 2). The temperature of the spring water changes little during the 12 months (44° to 54°). This water tends to maintain uniform temperature in the bog throughout the year, especially in the lower levels.

Temperatures of the profile were checked in other communities. Only the surface temperatures were found to vary from those of the above profile. The surface of a profile with a higher water table will show 1°–2° lower temperature than one with a lower table after both are exposed to the sun for several hours.

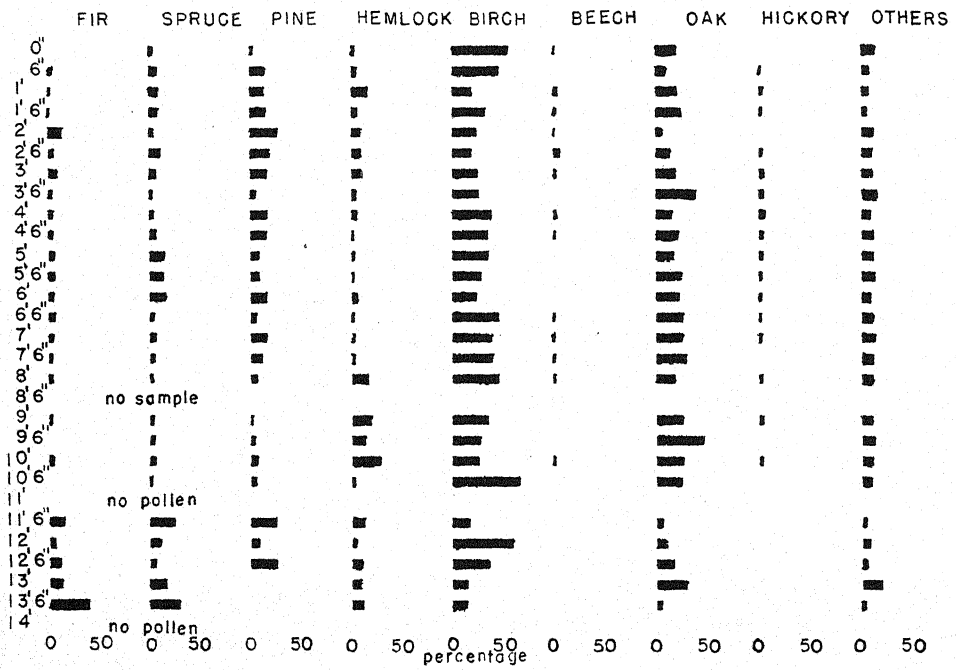


FIG. 13.—Pollen diagram at station 10, transect B. (Pollen analysis of samples by PAUL B. SEARS)

SOIL PH.—At station 10, transect B, the pH profile was also determined colorimetrically to a depth of 10 feet. The values, beginning at the surface, are: surface and 6-inch levels, pH 3.8; 1- and 2-feet, pH 4; 3-, 4-, and 5-feet, pH 4.4; 6-, 7-, and 8-feet, pH 4.6; 9- and 10-feet, pH 4.8. The peat, although slightly less acid at the lower levels, is still very acid at 10 feet. KURZ (5) found in Illinois bogs a decrease in acidity with increase in depth. This high acidity in the lower levels may be caused, as KURZ suggested, by sphagnum remains in the peat or by the downward movements of water. The pH of the surface layers was also determined in the various plant communities, and the results are given in the community descriptions.

POLLEN PROFILE.—At station 10, transect B (which is also station 16, transect D, at the center of Big Glade), samples of the sediments were taken at 6-inch intervals from the surface to the 14-foot level. The pollen diagram (fig. 13) shows the results. The $13\frac{1}{2}$-foot level shows a spruce-fir dominance. From that level to the surface the spectra are similar to the surface or present level, with the exception of the absence of fir at the surface level. The 12-foot level in marl and the 8-foot in algal ooze show little difference from the present level, indicating that all the sediments from the 13-foot level to the surface were deposited while the surrounding vegetation was similar to the present.

Vegetation

REGIONAL CLIMAX

The variation in altitude (3400–4600 feet) allows the persistence of three climaxes: northeastern coniferous or boreal forest, transition forest, and eastern deciduous forest. On Round Hill at 3550 feet a quadrat study shows the following, based on stem count: 70 per cent *Acer saccharum*, 4 per cent *Fagus grandifolia*, 10 per cent *Betula lutea*, 6 per cent *Prunus serotina*, 5 per cent *Tilia americana*, and 5 per cent *Prunus pennsylvanica*.

At 4100 feet on Cranberry Mountain the climax is a transition forest of yellow birch, sugar maple, beech, spruce (*Picea rubra*), and hemlock (*Tsuga canadensis*), with *Ostrya virginiana* as a smaller tree and *Corylus rostrata* as the most common shrub. A quadrat study shows 30 per cent *Betula lutea*, 33 per cent *Acer saccharum*, 20 per cent *Picea rubra*, 6 per cent *Fagus grandifolia*, 5 per cent *Tsuga canadensis*, and 6 per cent *Ostrya virginiana*.

Above 4200 feet the maple loses its dominance to spruce. On Kennison Mountain at 4500 feet a quadrat study shows 65 per cent *Picea rubra* and 30 per cent *Betula lutea*. *Abies fraseri* has not been found in this area with the spruce and birch, although in other parts of the state it does occur with these two species (3). *Taxus canadensis* is often found in this area as a low shrub. The range of mountains, including Black and Kennison, is known as "Yew Mountains" because of its abundance. The elevations where spruce dominates are somewhat lower than the 5000-foot lower limit in Tennessee and North Carolina, 400–500 miles farther south. Soil factors as well as climatic ones may have some influence on the zonal limits. *Populus tremuloides* and *Prunus pennsylvanica* have been observed as chiefly occupying a burned-over area.

VEGETATION OF BOG

The entire level area of 600 acres popularly called Cranberry Glades may properly be classified as bog. Peat covers most of the area, varying in thickness from a few inches to 11 feet. The entire

area is acid, ranging pH 3.8–6.2. The soil of all communities where sphagnum or other moss grows is near pH 4 and about pH 6.2 where no moss grows. All the area except a part of the shrub communities is partially covered with sphagnum, and there are a number of genera of the heath family, such as *Andromeda*, *Menziesia*, *Vaccinium*, and *Gaultheria*. The water table is within an inch of the surface most of the year and sinks to 12 inches below only during dry periods. This water in the partially decayed vegetable matter gives a quaking effect to the area wherever the peat is 5 feet or more in thickness. Carnivorous plants are represented by *Utricularia cornuta* and *Drosera rotundifolia*.

ORIGIN OF BOG CONDITIONS.—RIGG (7) mentioned two ways that bogs may develop: (*a*) in lakes and (*b*) in swamps. The popular belief is that this area was once a large mountain lake. Suggestions of dam formation by beavers or by landslide have been offered as possible causes of the lake. As the work progressed, this theory appeared inadequate for explanation of the existing conditions. During the last year of study, after checking the levels of the area, the lake theory of origin was abandoned as improbable if not impossible for three reasons: (1) The elevation of the lower end of the bog area is 45 feet lower than the upper end, at both the surface and the bottom of the peat, and the width of the area at the lower end is 400–500 feet. A dam 45 feet in height and 500 feet in length would have been required to flood the entire area, the construction of which would have been beyond the power of beavers or such landslides as could have occurred here. (2) If the whole area had been flooded, the deepest peat and certainly the deepest algal ooze and sedimentary peat should be found toward the lower end of the area; this is not the case, for the deepest peat and algal ooze is toward the upper end (figs. 6, 8). (3) If the origin had been in a lake, the peat deposits should cover the whole area, even under the streams. The profiles show chiefly inorganic sediments under all the streams. The peat accumulations thin out toward the streams and do not extend under them.

Another theory, which will better explain the existing conditions, attributes the damming to stream action. The maps and profiles (figs. 2, 3, 5–10) show that the main peat accumulations are located in the angles of the junctions of the streams. This led the writer to the theory that the conditions giving rise to peat accumulation owed their origin to the streams. Apparently the whole area, described previously as a part of the Harrisburg peneplain at the close of Tertiary time, was 10–15 feet lower than at present. It had been widened and base-leveled by the then longer streams while the river farther down was cutting in much harder rocks. The slope of the area may at that time have been less than the 45 feet in 3 miles shown at present. Following this period there came a change from degrading to aggrading action, and inorganic sediments were deposited as levees along the stream courses, thus damming areas away from the streams in which water could accumulate. Some explanation must be found for the change from degrading to aggrading action. This could have happened possibly during the later part of the glacial period, when increased precipitation, decreased evaporation, and lesser vegetation cover on the slopes caused the swollen streams to be overloaded with sediments, which were deposited over the level area. The coarser sediments would be deposited nearer the stream courses as levees, and the finer

clays would settle out later in the ponded water in the depressions away from the streams. The marl and algal ooze may have been deposited during this period while the climate was too cool for many aquatic seed plants. During the post-glacial period the levee building continued, but more slowly because of less precipitation, more evaporation, better vegegation cover, and a shortening of the streams through further piracy by Stamping Creek. The coming of a warmer climate speeded up plant growth in the ponded water, filling it with peat and initiating the sedge swamp.

Figure 3 and the transect profile A (fig. 5) show that the old channel of Cranberry River was building a much wider levee on its left bank. This was due to a bend in the stream to the right, so that most of the overflow was on the left. This bend also was one probable cause of the change of course later to the south side of the levee and along the margin of the peat accumulation in Big Glade, explaining the wide extent of inorganic sediments now on the right bank of Cranberry River and its erosion into peat on its left bank, both of which had puzzled the writer for several years.

Levee building, which thus probably occurred through part of the glacial and post-glacial periods, has now probably ceased in most parts of the bog. Cranberry River has recently cut through the very resistant Princeton conglomerate and is now cutting in the soft Hinton strata for several miles below the glades. Since the stream there has a drop of almost 100 feet to the mile, it should cut down rapidly, thus rejuvenating the streams of the glades. There is evidence that this is occurring in the recent shifting of Cranberry River from its old meandering channel to a nearly straight course along the side of Big Glade (fig. 3).

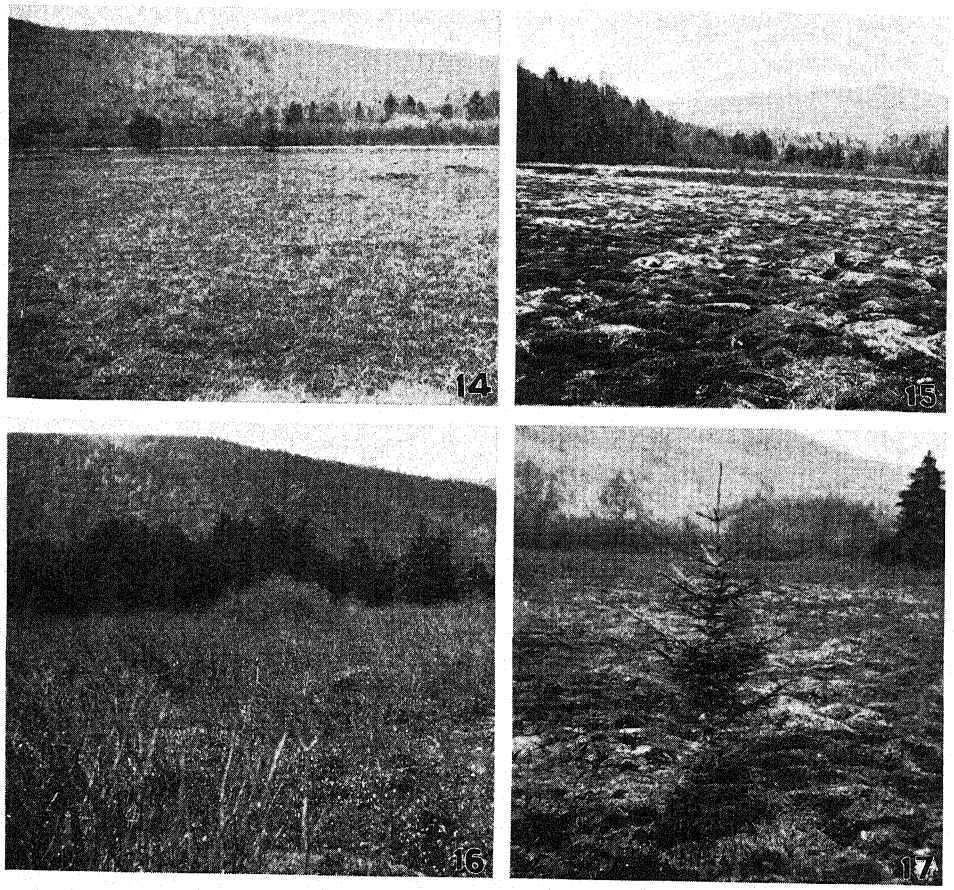
This theory explains all the conditions existing at present, including the sink in Big Glade referred to earlier. The bottom of peat here is 8 feet lower than the bank of Charles Creek. This could have been a former stream bed, 3–4 feet lower than the surrounding area, permitting a deeper ponding of water. The profiles show that the present surface of peat in some of the glades is higher than the levees which assisted in its origin. This is the condition of the "raised" bog (7). Once peat accumulation is started, it may form its own dam owing to slow movement of water through it and can develop on a slope, especially in cool climates (4). Parts of Flag Glade on transect D show this condition, as do also parts of Big Glade. The levees produced the undrained condition which was necessary to start bog development.

SHRUB COMMUNITIES.—These communities cover approximately 400 acres and extend as a wide belt along the streams and around the margin of the glades (figs. 4, 14). On the basis of soil, moisture, and floristic composition two units may be recognized, designated the *Alnus-Sambucus* and *Alnus-Viburnum* communities.

The *Alnus-Sambucus* community occurs on the levees or floodplains near the streams, where the water table varies from 1 to 30 inches below the surface. The soil is alluvium with some humus. The soil reaction is pH 6–6.2. The chief shrubs are the alder and elder, although some others are found. A quadrat study shows 70 per cent *Alnus incana*, 20 per cent *Sambucus canadensis*, 2 per cent *Ilex monticola*, 2 per cent *Viburnum dentatum*, 3 per cent *Taxus canadensis*, and 3 per cent *Crataegus* sp. In some parts of the community scattered trees occur, including *Fraxinus nigra*, *Prunus sero-*

tina, P. pennsylvanica, Betula lutea, Tsuga canadensis, and *Picea rubra.*

Many herbaceous plants occupy the ground stratum of this community, their societies giving seasonal aspects to it. *tus, A. prenanthoides, Trautvetteria carolinensis, Aconitum uncinatum,* and *Chelone glabra.* As a part of the aspection should be included woolly aphids on *Alnus incana,* forming great white masses



FIGS. 14–17.—Fig. 14, *Sphagnum-Vaccinium-Rynchospora* community with sedge, shrub, bog forest, and climax forest of Kennison Mountain in distance. Fig. 15, *Polytrichum-Cladonia* community in Big Glade with patch of *Andromeda glaucophylla* and shrub belt along Charles Creek. Round Hill is on left and Kennison Mountain on right. Fig. 16, shrub and bog forest invading *Polytrichum-Cladonia* community in Flag Glade. Shrub is chiefly *Viburnum cassinoides,* which aids in hummock formation. Kennison Mountain at back. Fig. 17, young *Picea rubra* in *Polytrichum-Cladonia* community. This spruce, 5 feet in height, had horizontal roots 9 feet long between moss mat and peat surface.

The vernal societies include *Claytonia caroliniana, Viola cucullata,* and *Ranunculus septentrionalis.* The estival societies include *Phlox maculata, Thalictrum* sp., *Veratrum viride, Ribes prostratum, Senecio aureus,* and *Carex scoparia.* The autumnal aspect is given by *Aster panicula-*

2 feet in length on the branches and visible at half a mile. These occur through June, July, and August.

The *Alnus-Viburnum* community is found around the margins of all the glades. The elevation is about 12–18 inches lower than the *Alnus-Sambucus*

community, and the water table varies from 2 inches above the surface to 7 inches below. Surface soil reaction is pH 4.4. *Sphagnum palustre* grows over much of it, and there is an accumulation of peat or organic material to a depth of from a few inches to 5 feet. The total area is greater than that of the *Alnus-Sambucus* community. A quadrat study shows 40 per cent *Alnus incana*, 25 per cent *Viburnum cassinoides*, 7 per cent *Ilex verticillata*, 8 per cent *Amelanchier oligocarpa*, 8 per cent *Pyrus melanocarpa*, 4 per cent *Pyrus americana*, 5 per cent *Menziesia pilosa*, and 3 per cent *Hypericum densiflorum*. Seasonal aspects also occur under the shrubs in the vernal societies of *Caltha palustris*, *Symplocarpus foetidus*, *Ranunculus septentrionalis*, and in the estival societies of *Polemonium van bruntiae*, *Arisaema triphyllum*, *Thalictrum* sp., *Oxalis acetosella*, *Osmunda cinnamomea*, *O. regalis*, *Rosa carolina*, and *Sium cicutaefolium*. The main factor differentiating these two communities is the difference in the depth of water table and possibly the amount of organic material of the soil. The difference in acidity is due chiefly to sphagnum, which is absent from one community.

CAREX-SPHAGNUM COMMUNITY.—This community extends around the eastern and western margin of Big Glade and the eastern margin of Flag Glade. It also occupies most of Round Glade, the upper half of Long Glade, and the margins of the remainder of Long Glade (figs. 4, 14). The main species of sedge is *Carex rostrata*, which extends throughout the community and makes up at least 90 per cent of the total sedge population. In some parts of the community rather extensive stands of *Carex trisperma*, *C. scirpoides*, and *C. lurida* occur. The sphagnum here is mostly *S. capillaceum*, with some *S. palustre*. This community includes also more or less of *Rubus hispidus*, *Vaccinium macrocarpon*, and *V. oxycoccus*. Little aspection is obvious after growth of sedge begins in the spring.

From 1 to 6 feet of peat underlie this community. The water-table level and pH value, 4.4, are close to those of the *Alnus-Viburnum* community, which borders it and is invading it, and to those of a *Sphagnum-Vaccinium-Rynchospora* community, which also borders it. In some places *Carex rostrata* has encroached upon the latter community as much as 15 feet in 8 years through growth of its underground rootstocks. In other places the ecotone of the two communities has not changed in 8 years; in others sphagnum has spread into pure sedge areas. Since 1–2 feet of sphagnum peat overlies 4–5 feet of sedge peat in much of the glades, sphagnum must have grown upon and killed out the sedges which first formed the peat. It is difficult to understand how sphagnum could kill out *Carex rostrata*, since the latter can invade sphagnum areas and by means of its rootstocks adjust itself to any building up of peat by sphagnum. The sedge or sedges which first formed the peat may have been acid-intolerant, existing for a long period in the absence of sphagnum and acid. With the invasion of sphagnum and the induced change in environment, they succumbed.

SPHAGNUM-VACCINIUM-RYNCHOSPORA ASSOCIATION.—This community includes a total of 50 acres in the upper and lower ends of Big Glade, the lower half of Long Glade, the upper one-third of Flag Glade, and a part of central and western Round Glade (figs. 4, 16). The percentages of each of the components of this association would be difficult to determine. *Sphagnum capillaceum*, *S. palustre*, *Vaccinium oxycoccus* (small cranberry), and *Rynchospora alba* (beaked rush) are present throughout the area. *Rubus hispidus* and *Vaccinium ma-*

crocarpon may or may not be present and in varying abundance. Through the summer the beaked rush gives the meadow aspect, which is modified in some parts by an abundance of two species of orchids (*Pogonia ophioglossoides* and *Calopogon pulchellus*); the autumnal aspect is given by *Eriophorum virginicum* (cotton grass), with large cotton-like masses of fruit. Cotton grass extends more or less through all the communities, giving the autumnal aspect to the whole area except the shrub and bog forest communities. *Aster umbellatus*, *Dulichium arundinaceum*, and *Epilobium densum* also add to the autumnal aspect of this association in Long Glade and in some other parts.

The reaction of the soil is pH 4. The water table varies from 2 inches above the soil surface to 7 inches below during droughts. Much of the time the water table is at the surface of the partially decayed sphagnum material, permitting the live sphagnum to be filled with water continually. The water-table level is one of the main factors determining this community, which is always located in the poorly drained areas. The upper end of Big Glade is nearly level and receives water from seepage, springs, and small streams from Cranberry Mountain; the lower end is nearly level and has its water table raised somewhat by overflow from Charles Creek, which is more than 1 foot higher. The upper end of Flag Glade is almost level and receives water from overflow and seepage from Yew Creek and springs from the hills. The lower half of Long Glade is lower than the levee or floodplain of the old channel of Cranberry River, which extends close to the protruding hill on the west and cuts off drainage by its levee at that point (fig. 3). Soil temperature is possibly another factor in determining this community. Temperature at the surface is always 1°–3° lower than in the *Cladonia-Polytrichum* community. This difference in temperature is partly due to the higher water table and partly to the influence of cold springs. This association is being encroached upon by *Carex rostrata* in some places and by *Polytrichum strictum*, *P. ohioense*, and *P. commune* in others—where the water table is being lowered.

POLYTRICHUM-CLADONIA ASSOCIATION.—This community is limited chiefly to Big Glade and Flag Glade and occupies about two-thirds of each. It occurs in the part of Big Glade between the *Sphagnum-Vaccinium-Rynchospora* association on the east and west sides of this glade and also in the northwestern part of Flag Glade (figs. 4, 15). The total area is 50–60 acres. This association includes *Polytrichum strictum*, *P. ohioense*, *P. commune*, *Cladonia rangiferina*, and *C. tenuis*. There may be more or less of *Rubus hispidus* and *Eriophorum virginicum*, the latter giving the autumnal aspect to the association. The aspect changes with the development of lichens. During periods of rainy weather and high water table, *Cladonia* grows very rapidly, spreading over whole clumps of *Polytrichum*, so that much light gray color is mixed with the green. During long periods of drought the lichen is deprived of water and dries so that it can be crumbled to powder, while the moss with long stems extending to the moist peat or organic soil then will grow around and over it.

The reaction of the soil surface in this community is pH 3.8, increasing somewhat at lower levels. The water table is 3–6 inches lower than in the communities surrounding it. The lower water table and the lower pH have the same cause. The association in Big Glade is higher than Cranberry River on its north, and it lies along the north side of Round Hill. This deprives it of any water from seep-

age, springs, or streams, leaving rainfall its only source—which is void of mineral bases capable of neutralizing the acidity. The *Sphagnum-Vaccinium-Rynchospora* association at the upper end of this glade receives the same amount of rainfall and in addition some water from the hills, thus producing a higher soil reaction and a higher water table. Similar conditions exist in the *Polytrichum-Cladonia* association in Flag Glade, also occurring on "raised" peat. It is higher than Charles Creek on its east and slopes toward the hill on the west and so is deprived of telluric water and bases, resulting in a low pH and a low water table. Wind sweep may also have some effect in determining this community. The central part of Big Glade and lower part of Flag Glade are most exposed to the winds moving through the valley. This would tend to lower the water table and increase transpiration, favoring *Polytrichum* rather than sphagnum.

The encroachment of sphagnum and *Polytrichum* upon each other is determined by the height of water table, by the amount and frequency of rainfall, and by the rate of evaporation. During periods of much rainfall and high relative humidity, sphagnum will grow over *Polytrichum*, cutting off its light and killing it. In many parts of the association this competition may be observed through the season. Where the water table is being lowered, as along Cranberry River on the north side of Big Glade, *Polytrichum ohioense* and *P. commune* are encroaching upon the *Sphagnum-Vaccinium-Rynchospora* association.

Three colonies of *Andromeda glaucophylla* have become established in the *Polytrichum-Cladonia* association in Big Glade. One large and two small colonies cover nearly an acre (fig. 15). It has greatly increased its area in 10 years, suggesting that it was introduced by bird dissemination of seeds from some other bog. A similar case is found in the *Sphagnum-Vaccinium-Rynchospora* association, where two colonies of *Menyanthes trifoliata* have established themselves, one in Long Glade and one in Big Glade, and have spread greatly in 10 years. If they have been here for a long period, they should be more widely distributed over the area.

The *Polytrichum-Cladonia* community is being encroached on all sides by shrubs from the *Alnus-Viburnum* community. *Hypericum densiflorum* has driven a wedge into the association from Cranberry River on the north side of Big Glade. *Pyrus melanocarpa*, *Ilex verticillata*, and *Viburnum cassinoides* are scattered through much of the association.

Hummocks are found in many parts of this association, usually with the shrubs (fig. 16). As mentioned before, wind movement is strong, increasing evaporation, so that at times in the summer the water table descends to 12 inches below the surface of the soil. Growth of *Polytrichum* is thus slowed down many hours and days during the summer. Those moss plants growing under a small shrub with its protective canopy of leaves, such as *Viburnum cassinoides*, will remain more moist, with resultant longer growing periods, so that they elevate themselves above the surrounding moss. *V. cassinoides* can adjust itself to the upward growth of the moss by developing adventitious roots. One plant was observed with such roots 18 inches above the original ones. Other shrubs than *V. cassinoides* also assist in hummock formation. After a hummock is once started, the shrub may die and the hummock continue to grow, mostly on its leeward side.

A study was made of the roots of many of the bog plants. Most of them were in

the *Polytrichum-Cladonia* association. None of the shrubs scattered through this association are able to penetrate the acid peat but extend their roots horizontally on the surface or above it in the living moss mat. A specimen of *V. cassinoides* 3 feet in height had four horizonal roots 8–9 feet in length. *Hypericum densiflorum* 12 inches high had one horizontal root 6 feet long and two shorter ones. *Andromeda glaucophylla*, *Pyrus melanocarpa*, and *Gaultheria procumbens* of similar heights had roots of corresponding lengths. A red spruce 5 feet in height had twelve horizontal roots 6–9 feet in length (fig. 17). None of these roots had penetrated the peat vertically more than 1 inch. The plants are held up by the moss and could not exist without it. Only four of the species were able to penetrate the peat more than 1 inch. These were *Eriophorum virginicum*, *Rynchospora alba*, *Menyanthes trifoliata*, and *Carax rostrata*. One plant of *E. virginicum* 2 feet high had roots extending vertically almost 2 feet. As previously mentioned, it is found over the whole area except in the shrub and bog forest communities and gives the autumnal aspection to the glades. Vertical roots of *C. rostrata* were found up to 18 inches long. *R. alba* penetrates the peat 6 inches with several vertical roots. *M. trifoliata* in the *Sphagnum-Vaccinium-Rynchospora* association had roots 18 inches deep in the sphagnum material. It probably will extend its area in this association.

BOG FOREST ASSOCIATION.—This community, covering about 80 acres, is on the periphery of the entire bog area (figs. 14–16). Red spruce and hemlock are the most common species. A quadrat study shows 75 per cent *Picea rubra*, 12 per cent *Tsuga canadensis*, 8 per cent *Betula lutea*, 5 per cent *Fraxinus nigra*. The shrubs include *Ilex verticillata*, *Viburnum cassinoides*, *Rhododendron maximum*, and *Taxus canadensis*. Sphagnum grows over much of the forest floor. The soil reaction is pH 4.5. The water table is high, only slightly lower than in the *Alnus-Viburnum* community, which forms an irregular ecotone with the bog forest and is being encroached upon by it. The soil is organic and varies in depth from 1 to 5 feet of peat.

In addition to invasion of the *Alnus-Viburnum* community, the bog forest is also invading the moss-lichen and sphagnum-cranberry-beaked rush communities, mostly in Flag Glade and Round Glade. Spruce is the only tree species invading the moss communities, except one specimen of *Pinus strobus* and three or four of *P. rigida* which have attained a diameter of 6–8 inches in Big Glade. It is very difficult for these trees to invade the moss communities, since their roots cannot penetrate the peaty soil and must run horizontally under the moss, so that they are soon casualties to the strong wind-sweep in the glades. If they can develop in a group of four to eight, they support one another. Many such groups occur in Big Glade and in Flag Glade, but show a slow rate of growth.

A study was made of the rate of growth of young spruce on Kennison Mountain, in the bog forest, and in the moss-lichen community of the open glade. Trees 2.5 inches in diameter were cut 1 foot from the soil, and annual rings were counted. The specimen from Kennison Mountain showed an age of 19 years; the one from bog forest, 49 years; and the one from the moss-lichen community, 31 years. The bark was equal in thickness on the mountain and bog forest specimens but was more than twice as thick on the glade specimen. The tree in the bog forest had two important limiting factors—difficulty of absorption and lack of sunlight. The one in the open glade was limited in absorption of water

and nutrients and was exposed to strong winds. With equivalent rate of growth, some of the spruces in the clumps in Big Glade 8 and 10 inches in diameter would be 125 years or more in age.

Only a few spruce seedlings are encountered in the open glade, showing that establishment there is very difficult (fig. 17). The migration is chiefly from the west, in the direction of the prevailing winds, which carry the seeds. The recently uprooted spruces in the glades, the trunks now being covered with mosses and lichens, and the countless trunks encountered at all depths to 13 feet in the borings, all attest the attempts at invasion of this area for hundreds of years, perhaps thousands, and the magnitude of the casualty list.

Discussion

The present study has revealed much about the origin of the level area and the origin of the bog conditions, yet there are many questions unanswerable relative to past history and age. It is very difficult to estimate the length of time represented by the 15 feet of sediments which have been studied. They are certainly of Quaternary origin. They probably represent the later part of the glacial and post-glacial periods.

The pollen diagram shows that spruce and fir were dominant regionally at the time of the accumulation of the 13½-foot level of marl (fig. 13). This might seem at first to make the bog comparable in age with the Ohio and Indiana bogs, which are estimated at 30,000 or more years (6), and showing the same forest sequences mentioned by SEARS (9). The 13½-foot level would thus represent the cool-moist or spruce-fir period; the 11–12½-foot levels, the warmer and drier or pine period, etc. The altitude here is 2800–4000 feet higher than at the Indiana and Ohio bogs, however, and the spruce-fir dominance undoubtedly persisted longer. The time since spruce-fir dominance in those areas has been estimated at about 10,000 years (8). The rate of peat formation has been the basis for this estimation (10). In the area of this study the rate of peat formation has probably been slower than in the other bogs mentioned because of lower temperature and differences in origin and continuation of bog development.

The clays and marl probably were deposited in the later glacial period, and perhaps a part of the algal ooze was formed then—or in the early post-glacial period while it was yet too cool for many aquatic seed plants. Most of the peat probably was formed in the post-glacial period.

When the depressions were first formed by the levee building of the streams, water accumulated, especially in the Big Glade area. For a long period it was too cold for much plant life, so that clays and marls and later algal ooze accumulated; then, as the climate became warmer, increased plant life filled the water and initiated the sedge swamp, which formed the 5 feet of sedge peat now overlying the 4 feet of algal ooze and sedimentary peat. Some shrubs were scattered through the sedge (*Hypericum densiflorum*, for example), remains of which are now found in the sedge peat. The sedge community later retrograded to sphagnum over most of the glade area. Sphagnum has recently been giving way to *Polytrichum* in the more raised and better drained parts.

Trees have grown in the bog area since the sedge swamp was initiated and probably for some time before. Logs of spruce and hemlock are encountered to the bottom of the peat. Those in sedimentary peat or algal ooze could have been washed in by stream overflow. No logs have been encountered below the 13-

foot level. Shrubs have probably been growing along the streams since the time of deposit of the 13-foot level of sediments.

The future of the area with its successions and time required is as difficult to surmise as is its past. Succession is taking place, as shown by observations over the last 10 years. Shrubs are encroaching upon the moss-lichen, the sphagnum-cranberry-beaked rush, and the sedge-sphagnum communities. Trees are encroaching upon the shrub and other communities. Where shrubs or trees persist, their leaves and needles accumulate to raise the level higher above the water table and the acid substratum. At present, where a spruce or a clump of spruces have established themselves, many shrubs—such as *Menziesia pilosa*, *Viburnum cassinoides*, *Pyrus melanocarpa*, and *Gaultheria procumbens*—are thriving and spreading out from this area into the moss-lichen community.

In 1934 stakes were set up at a number of places on the border of different zones where encroachment seemed probable. During the succeeding 8 years *Gaultheria procumbens* has spread 10 feet in all directions from a clump of spruces into the *Polytrichum-Cladonia* association, or 1½ feet per year. *Pyrus melanocarpa* advanced 25 feet, or 3 feet per year, into the same association. *Hypericum densiflorum* advanced 17 feet from the shrub belt on Cranberry River at transect B into the moss-lichen community. An old settler, who had often visited the area years ago but not in the last 20 years, stated that 30 years ago there were no shrubs in the Big Glade but only moss-lichen "tundra" with cotton grass and beaked rush. He was much surprised in 1941 to see the encroachment by shrubs from all sides. *Pyrus melanocarpa* is making more headway than any of the other shrubs. It has an underground rootstock which grows rapidly under or through the moss layer and sends up new shoots. Yet its roots cannot penetrate the peat and it depends upon the moss for anchorage. It will not be able to survive without the moss until the soil has been built up by the decay of leaves and other plant parts, or better drainage is otherwise provided.

At another staked area, *Carex rostrata* advanced upon a sphagnum-cranberry-beaked rush community about 15 feet during the 8 years. The sedge has underground rootstocks which provide a good mechanism of encroachment similar to that of *Pyrus melanocarpa* and *Gaultheria procumbens*. Sphagnum spread several feet into a pure *Carex rostrata* community and an *Alnus-Viburnum* community. It can spread rapidly owing to its ability to absorb water and minerals through any part and to propagate vegetatively from many of its parts.

The bog forest is encroaching on every side of the area, but mostly on the west, the direction of the prevailing winds. Dispersal of conifer seeds should be greater from the west, and the young trees are better protected from excessive evaporation and uprooting.

It is only a question of time until the shrubs will cover the whole 600 acres, gradually to be replaced by bog forest. Once it is established, it should remain as an edaphic climax, until the streams are entrenched so that the peat can be eroded and provide a drained soil for the regional climax forest. The rate of downward cutting of the streams is greatly increasing, owing to the fact that Cranberry River, which has been cutting in the hard Princeton conglomerate below the bogs, is now cutting in the soft Hinton strata. The new course of the river along the north side of Big Glade is evidence that entrenchment and peat erosion are now occurring.

Summary

1. The Cranberry Glades area in West Virginia comprises about 600 acres of apparently level land covered with bog forest, shrub, and areas of sedges, mosses, and lichens, commonly called "glade." Most of the area is underlain by peat or organic material from a few inches to 11 feet in thickness.

2. The level area, upon which 15 feet of inorganic material and peat have accumulated, represents a part of the Harrisburg peneplain temporarily base-leveled in the soft shales and sandstones of dipping strata.

3. Bog conditions were initiated by the deposition of levees along the banks of streams, causing undrained conditions in the areas away from the streams. This change from degrading to aggrading action probably occurred during glacial time, when the streams, swollen by increased precipitation and decreased evaporation and overloaded with sediments from slopes unprotected by sufficient vegetation cover, deposited their sediments over the area. In post-glacial time the streams with reduced volume of water slowly built their levees higher during floods, causing further damming and further peat accumulation.

4. Six plant communities are differentiated: sedge-sphagnum, sphagnum-cranberry-beaked rush, moss-lichen, bog forest, and two shrub communities.

5. The structure and pollen of the transect profiles of the sediments and the existing communities point to succession from shallow water, with its accompanying communities, to a sedge swamp, to a retrogression to sphagnum, to moss-lichen in some parts, to shrub, to bog forest.

6. Succession will continue until bog forest has occupied the whole area, to be followed by regional climax forest only when sufficient stream erosion has removed the peat accumulation and provided sufficient drainage.

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LITERATURE CITED

1. BROOKS, A. B., Cranberry glades. West Virginia Geol. Surv. 5:358–368. 1911.
2. ———, Native trees and shrubs of West Virginia. West Virginia Geol. Surv. 5:368–427. 1911.
3. CORE, E. L., Plant ecology of Spruce Mountain. Ecology 10:1–13. 1929.
4. IVES, R. L., Atypical subalpine environments. Ecology 23:89–92. 1942.
5. KURZ, HERMAN, Influence of sphagnum on bog reactions. Ecology 9:56–69. 1928.
6. POTZGER, J. E., Pollen spectra as time markers. Amer. Mid. Nat. 25:224–227. 1941.
7. RIGG, G. B., The development of sphagnum bogs in North America. Bot. Rev. 6:666–693. 1940.
8. SEARS, P. B., The archaeology of environment in eastern North America. Amer. Anthrop. 34: 610–622. 1932.
9. ———, Forest sequences in the north central states. BOT. GAZ. 103:751–761. 1942.
10. SEARS, P. B., and JANSON, ELSIE, Rate of peat growth in the Erie Basin. Ecology 14:346–356. 1933.
11. SHELDON, J. L., An annotated list of fungi destructive to forest trees and woods. West Virginia Geol. Surv. 5:82–86. 1911.
12. STRAUSBAUGH, P. D., Cranberry glades. Amer. Forests 40:362–364; 382–383. 1934.

SURVEY OF ANATOMY, ERGASTIC SUBSTANCES, AND NUCLEAR SIZE IN ECHINOCYSTIS MACROCARPA AND CUCURBITA PEPO

FLORA M. SCOTT

(WITH TWENTY-THREE FIGURES)

Introduction

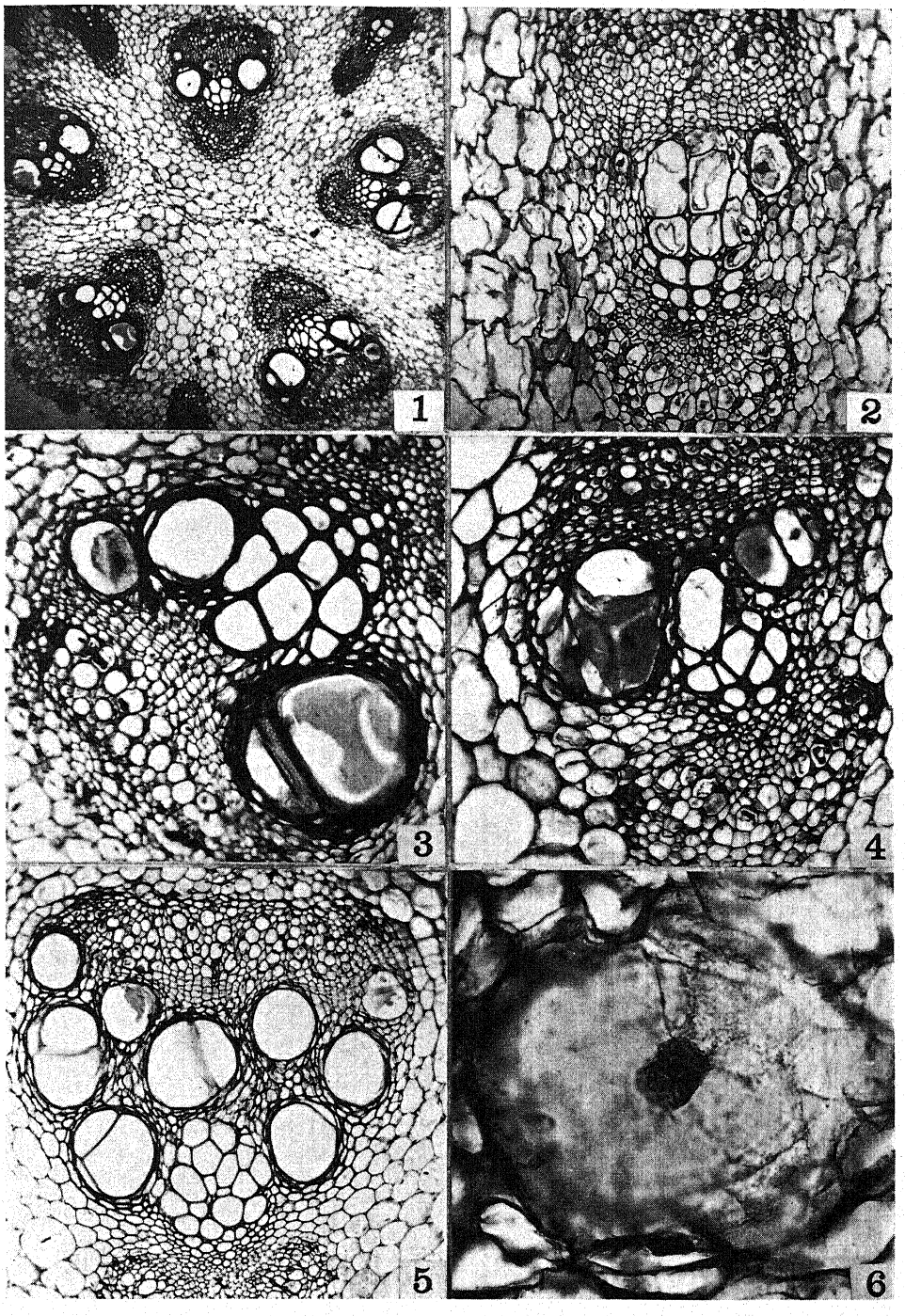
In the course of anatomical and cytological studies of *Ricinus communis* (8, 9), the lack of detailed knowledge of nuclear size in differentiating xylem vessels was apparent. In contrast to nuclei measuring 59,550 cu. μ in *Ricinus*, nuclei measuring 330,023 and 163,643 cu. μ, respectively, are present in the developing vessel segments of *Echinocystis* and *Cucurbita*. Nuclear size in other tissues is surveyed for the sake of comparison, and, since apparently no report has appeared on the subject, the anatomy of *E. macrocarpa* is outlined.

Material and methods

Echinocystis macrocarpa and *Cucurbita pepo*, of the family Cucurbitaceae, are vigorous scrambling or trailing vines (7). *Echinocystis*, a perennial, common in the chaparral of southern California, has an immense, completely buried storage tuber. In the first two seasons' growth this "root" is about the size and shape of a small carrot. When fully grown it is rough and gnarled on the surface and may measure 20–40 cm. in diameter, 50–90 cm. in length, and may weigh 40–60 lb. In early spring, one to eight vigorous shoots appear above ground, grow rapidly to a length of 60–90 cm., then branch profusely. As the lateral branches develop, secondary thickening in the main axis is most active. During this stage, which is of relatively short duration, differentiating vessels are present in every internode of the main axis. By the time of flowering the majority of the vessels are mature and without nuclei. Full-grown shoots may measure 250 cm. or more. Lateral branches continue to grow throughout the early summer, but since they are of smaller diameter, they are much less favorable for nuclear study.

Sections of fresh material of both *Echinocystis* and *Cucurbita pepo* (variety Kentucky) were cut from the younger and older tissues of shoot, root, and developing fruit and mounted directly in mineral oil or in water containing traces of vital stains such as neutral red or thionin. Nuclei were measured immediately. No trace of shrinkage is indicated in oil mounts for several hours, but in water, surface wrinkling of the nuclear membrane may occur within a few minutes or after a longer period. Permanent slides, necessary for tissue detail, were prepared by fixation in Navashin's solution followed by Delafield's haematoxylin or safranin and fast green stain. Feulgen's reagent was also used to intensify the chromatin network.

The percentage water by volume in the giant nuclei is estimated by observing volume contraction during and after irrigation of the section with absolute alcohol (9). Centrifugation of tissues, followed by immediate killing and fixing, results in a shrinkage even more pronounced. Accurate measurement in this case is impossible, however, because of the piling up of plastids and cytoplasm around the shrunken nucleus.



FIGS. 1–6.—Transections: Fig. 1, young stem; two or more large secondary vessels differentiated in inner bundles. Fig. 2, young bundle; left and right of primary spiral vessels (still unlignified) lie two secondary vessels, one small and one large, in early stage of differentiation. Figs. 3, 4, bundles; different stages of differentiation. Fig. 5, mature bundle; vessels empty, none tylotic. Fig. 6, differentiating vessel; giant nucleus already somewhat contracted.

Observations

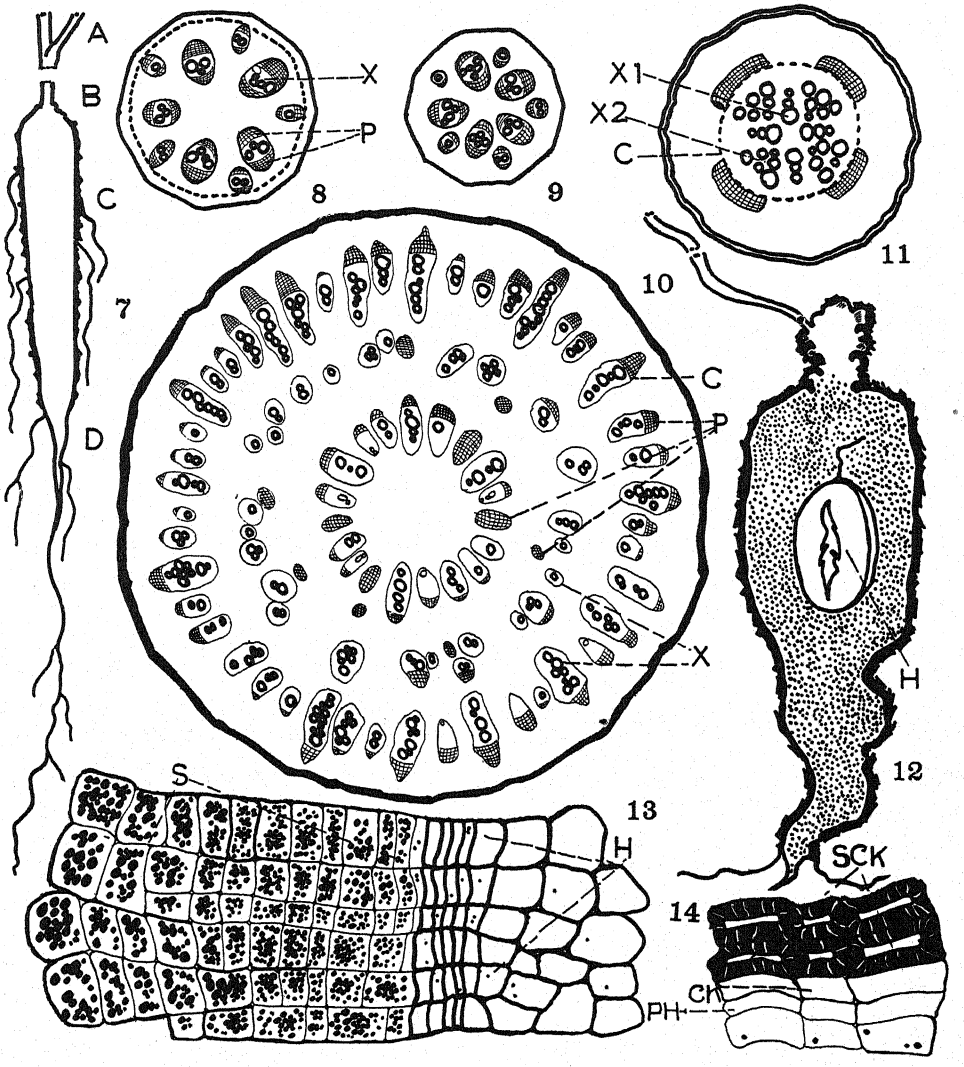
A. Echinocystis macrocarpa

Shoot.—The structure of the shoot resembles essentially that of *Cucurbita* recently summarized by Hayward (4). The entire surface, including buds, leaves, tendrils, and inflorescence primordia, is closely covered with pointed trichomes of varying length and by capitate glands. The developing axis, phyllotaxy 5/13, may be divided anatomically into four regions: (*a*) The apical meristem, surrounded by leaf and tendril primordia, is followed by twelve differentiating internodes 0.05–0.2 cm. in length. (*b*) In the zone of primary growth the internodes expand and elongate rapidly and measure approximately 0.3, 0.5, 1, 1.5, 2.5, 4, 6, 11, 18, 15, 17, 17 cm. The stem as seen in typical transection consists of epidermis, collenchyma, chlorenchyma, pericycle, and nine or ten bicollateral bundles arranged in two concentric rings in a ground tissue of ray and pith parenchyma. The primary xylem strands consist of five to seven radial rows of spiral vessels (figs. 1, 2), which are followed by scalariform and reticulate vessels. (*c*) About the level of the twenty-fifth internode the young stem reaches its maximum diameter and the lateral branches their maximum length. Secondary vessels, two or more per bundle, made up of very wide, short, pitted vessel segments now differentiate. They may appear at the same time and keep pace in expansion and in wall lignification, but more generally the development is not contemporaneous. One vessel may be completely lignified while the second is beginning to expand (fig. 3). In the twenty-seventh and proximal internodes the solitary vessels typical of the distal region may be replaced by a group of two to four differentiating vessels in the same stage of growth (fig. 4). (*d*) The mature region of the stem is usually bereft of leaves and all but the remains of tendrils; it narrows toward ground level and below. While growth of the shoot as a whole progresses, a few secondary vessels continue to differentiate (fig. 5).

Region (*c*) affords the best material for study of the giant nuclei. In any cross-section, two to eight or more differentiating vessel segments may be observed. During vessel expansion the living cytoplasm contains innumerable vacuoles (figs. 6, 20*E*). Mitochondria are numerous and chloroplasts are present, usually parietal in position. Protoplasmic streaming is frequently observed. Wall thickening may be followed from a barely visible cellulose mesh to the completely lignified, mature pitted wall, and the end walls eventually disappear.

The leaves and tendrils of *Echinocystis* are essentially similar in structure to those of *Cucurbita* (4). Since nuclear size is conservative throughout the tissues, the latter are not described in detail.

Tuber.—Owing to lack of seedling material, the early stages of tuber development were not observed. The storage cylinder of a typical plant 2–3 years old measures about 15 cm. in length and 1.5 cm. in diameter (figs. 7–11). It tapers off above and below toward shoot base and tap root. Numerous lateral roots are present throughout the tuber length (fig. 7). Since the transition to root structure occurs near the base of the storage region, the tuber presumably develops from the hypocotyl and stem base (figs. 7*D*, 11). The surface is covered with a thin layer of cork followed by phellogen and periderm. The pericyclic zone consists of fibers and parenchyma. The vascular bundles are arranged in three roughly concentric series and are surrounded by starchy parenchyma. The



FIGS. 7–14.*—Fig. 7, diagrammatic longisection of young tuber (actual diameter 1.5 cm.); length of storage region 15 cm.; length of shoot (etiolated) below ground 5 cm. General structure of shoot at ground level (*A*), at junction of shoot and tuber (*B*), of midstorage region (*C*), and of transition region (*D*) are illustrated in figs. 8–11. Fig. 12, diagrammatic longisection of mature tuber with cracked central heart devoid of starch; actual diameter and length 26 cm. and 60 cm.; actual diameter and length of heart 10 cm. and 20 cm. Fig. 13, transection of junction of storage region and heart of tuber; cambium-like layers lignified; heart without starch. Fig. 14, transection of cork, sclerenchyma, phellem and phellogen.

* *c*, cambium; *ck*, thin phellem; *emb*, embryo; *end*, endosperm; *f*, funiculus; *h*, "heart" of storage tuber; *i*, integument; *n*, nucellus; *p*, phloem; *pc*, pericarp; *pr*, parenchyma; *ph*, phellogen; *s*, starch; *sck*, sclerenchymatous phellem, hard cork; *st*, storage tuber; *t*, tylosis; *x*, xylem; x_1, protoxylem; x_2, secondary xylem.

Xylem vessels are similar in structure throughout the tuber, except for the tyloses in the older elements (fig. 19C). Tylotic walls may show reticulate thickening. In transection the core of the tuber resembles heart wood in demarcation. It is amber in color and completely devoid of starch. Along three-eighths of its circumference, tuber and core are split apart, while the rest of the perimeter is marked by a cambium-like zone of cells, the walls of which are completely lignified. Further evidence of tissue contraction is seen in a central split within the heart wood itself (figs. 12, 13).

Seasonal shoots arise from meristem present in the narrow, irregular tuber neck. If the surface soil has been removed by erosion, chlorophyll may be present in the neck tissue.

Reserve material in the tuber includes starch, fat droplets, and protein material. Starch grains in cells adjacent to vascular strands are generally small, while those in the center of the rays (elliptical, spherical, or irregular in outline) may measure 40–65 μ in diameter (fig. 18). In young cells starch-containing leucoplasts usually encircle the nucleus, but later the grains are distributed throughout the cytoplasm. In the majority of the parenchymatous cells one or two fat droplets are present which stain brilliantly with Sudan III. In addition to these microscopically visible ergastic substances, colloidal protein is indicated by Millon's reagent. Phloem strands are particularly well defined. The paths of collateral vascular bundles and of independent xylem and phloem strands may be clearly compared and contrasted in two adjacent longitudinal slices of tuber, 3 mm. or less, treated with Millon's reagent or with phloroglucin and HCl.

RESERVE MATERIALS IN SHOOT.—As might be expected in short-lived tissues, the shoot contains no significant amounts of reserve at any time. Starch is evident in the chlorenchyma of the outer cortex; but in the inner cortex, rays, and pith it is negligible. Small granules of sieve-tube starch occur in the sieve tubes. Fat droplets, one or two in number, 1–4 μ in diameter, are usually present in parenchymatous cells. As in the tuber, protein is definitely and somewhat curiously localized. The deepest coloration appears in the sieve-tube contents, but cell walls also are heavily stained, in particular in collenchyma, pericycle, crushed phloem, and xylem. The actual protoplasts of the parenchyma, even after half an hour, remain only slightly stained. In tuber and in shoot, calcium oxalate is practically absent.

NUCLEAR SIZE IN SHOOT.—The nuclei of the isodiametric meristem cells are relatively large, 4 μ in diameter, 33 cu. μ in volume. Nuclear size is considered in reference to this basic volume, V = 33.[1]

In contrast to this, the giant nuclei of the differentiating vessel segments measure 20,000–300,000 cu. μ and therefore show as much as a 10,000-fold increase in volume. When immediate increase is considered, the reference point is necessarily the nucleus of a cambial cell, 432 cu. μ, or 13V. The direct growth from cambial nucleus to vessel nucleus is thus 763-fold only (table 1).

The parenchymatous elements of the tuber cortex, rays, and pith may approach the vessel segments in diameter and equal or exceed them in length. The nuclei are consistently smaller, however, with a maximum of 180,757 cu. μ (5,477V, in contrast to the previous figures).

Measurement in chlorenchymatous cells is practically impossible, since chloroplasts consistently surround the living

[1] Throughout this paper, V is used for volume instead of the letter X of the original papers.

nucleus. The largest nucleus noted, 6,186 cu. μ, indicates 187-fold volume increase. The volume frequencies for nuclei of parenchyma and vessel segments are

TABLE 1

RANGE OF NUCLEAR SIZE IN SHOOT OF ECHINOCYSTIS MACROCARPA IN: *A*, APEX AND INTERNODES 2–13 APPROXIMATELY; *B*, REGION OF EXPANSION AND OF DIFFERENTIATION OF SPIRAL VESSELS, ETC.; INTERNODES 14–26 APPROXIMATELY; *C*, REGION OF ACTIVE SECONDARY THICKENING; INTERNODES 27–30 APPROXIMATELY; *D*, MATURE REGION, OFTEN WITHOUT LEAVES, TENDRILS, OR BUDS IN LOWEST INTERNODES

Region of stem	Cell		Nucleus		Nucleolus diameter (μ)	Scale of increase
	Type	Dimensions (μ)	Diameter (μ)	Volume (cu. μ)		
A	Meristematic, dividing	8 (isodiametric)	4	33	2	33=V
	Vacuolating, dividing	13– 30 (isodiametric)	5– 9	81– 432	2– 3	2.4 – 13V
B	Epidermis	22× 13– 22× 22	8	267	3	8V
	Trichomes	19× 40– 20× 13	6	163	2	4V
	Glandular hairs "head"	12=radius of sphere quadrant	8	267	2	8V
	Collenchyma	95× 17–152× 28	11	771	3	23V
	Chlorenchyma	66× 38	22	6,186	5	187V
	Parenchyma of cortex, rays, pith	70× 51–123× 62	17–38	2,935– 28,670	3– 6	88 – 868V
	Spiral vessels	32– 38 (diameter)	17–22	2,935– 6,186	3– 4	88 – 187V
C	Epidermis	150× 20	9	432	3	13V
	Collenchyma	209× 22–228× 26	9–11	432– 771	3– 4	13 – 23V
	Chlorenchyma	47× 38– 76× 38	19	3,582	5	108V
	Pericyclic fibers	200× 25–700× 30	9–11	432– 771	3– 5	13 – 23V
	Parenchyma of cortex, pith, rays, xylem, etc.	95× 66–475×114	11–38	771– 28,670	3– 5	23 – 859V
	Cambium	47× 17– 47× 22 (transection)	5– 9	91– 432	2– 3	2.7– 13V
	Vessel segments	49–437 (diameter)	9–85	432–330,023	3–12	13 –10,000V
D	Epidermis	114× 15–228× 28	11	771	3	23V
	Collenchyma	209× 30	11	771	3	23V
	Chlorenchyma	47× 34–250× 32	9–11	432– 771	3– 3	13 – 23V
	Pericyclic fibers	456× 32–760× 32	13–19	1,200– 3,582	2– 4	36 – 108V
	Parenchyma of cortex, pith, rays, xylem, etc.	209× 30–320×160	11–41	771– 38,159	2– 6	23 – 1,156V
	Cambium	28× 11 (transection)	9	432	3	13V
	Vessel segments	96–285 (diameter)	28–70	12,095–180,757	3–22×19	366 – 5,477V

The remaining tissues include trichomes and epidermal cells which range from 4V to 23V, collenchyma elements 13V to 23V, and pericyclic fibers 13V to 108V. summarized in table 2. Maximal frequencies for the larger xylem elements occur at 12,095, 28,670, 55,992, and 96,762, and at 163,643 a lesser peak. This

series falls approximately into the V, 2V, 4V, 8V series of MONSCHAU (6), the significance of which is discussed later.

CYTOLOGY OF GIANT NUCLEI.—The giant nuclei of the vessel segments and of the parenchyma appear to be in the resting or early prophase condition and are essentially similar to those in *Ricinus* (9). A reticulum (fine or coarse) is visible in untreated nuclei and is accentuated but not altered in outline by Feulgen's reagent. The nucleoli are variable in number, size, and shape. The nucleolar material is heterogeneous, with minute refractive droplets, 0.5 μ or less in diameter, scattered irregularly throughout (fig. 20*A–E*).

Cytoplasmic streaming along cell walls and in radiating protoplasmic strands indicates that the living cells are not killed in the process of sectioning. Nuclei when first detected are practically invisible, since they are translucent and faint in outline. Change in refractive index is more or less immediate, and the nucleus becomes microscopically clear. That increased visibility may actually entail decrease in nuclear volume, perhaps as a result of the shock of sectioning, is evident in parenchymatous cells. In longitudinal section the cells in any one vertical file are equal in diameter and approximately equal in length. In cells where protoplasmic streaming is apparent, the nucleus is translucent and is generally outlined by a sheath of chloroplasts. In the cells where protoplasmic streaming can no longer be detected, the nucleus is perfectly visible, and the halo of chloroplasts, perhaps slightly disturbed, now lies more or less removed from the nuclear surface. Table 3 shows that the diameters of living nuclei measure approximately twice those of nonliving.

Re-examination of earlier figures indicates that the variability in nuclear size in parenchymatous cells of approximately the same dimensions is doubtless due to this contrast between living and nonliving units.

Extreme nuclear contraction may be induced, as in *Ricinus*, by dehydration with absolute alcohol (8). When fresh sections are irrigated with alcohol, nuclear shrinkage begins immediately on penetration of the reagent and continues until equilibrium is reached. This minimum volume remains unaltered by addition of xylene. The percentage decrease in volume may be taken to indicate the percentage volume of "free" water. As the abbreviated figures in table 4 show, the amount varies from 39 to 82 per cent. The contracted nuclei are still much larg-

TABLE 2

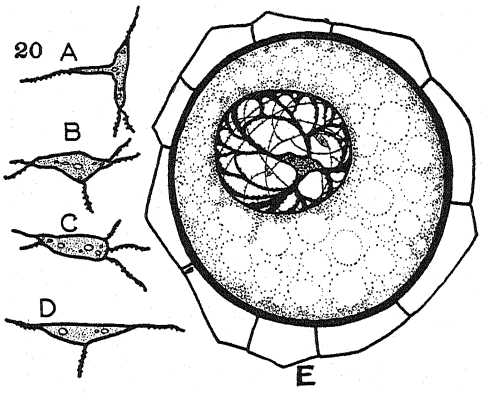
VOLUME FREQUENCIES OF NUCLEI IN XYLEM AND IN PARENCHYMA OF ECHINOCYSTIS MACROCARPA

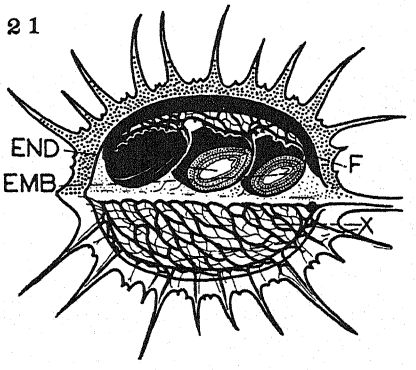
VOLUME (CU. μ)	FREQUENCY		VOLUME (CU. μ)	FREQUENCY	
	Xylem	Parenchyma		Xylem	Parenchyma
91...	.....	3	24,578...	1	.....
229...	.....	14	28,670...	48	10
432...	.....	27	33,327...	8	1
771...	4	36	33,433...	2	.....
1,200...	4	36	38,159...	15	.....
1,832...	10	22	43,601...	13	5
2,935...	10	15	44,600...	1	.....
3,582...	7	23	49,541...	1	.....
4,773...	10	11	55,992...	64	3
6,186...	18	17	62,980...	3	1
7,890...	21	9	70,521...	9	.....
9,605...	3	.....	79,081...	8	.....
9,827...	7	.....	87,403...	1	.....
12,095...	34	14	96,762...	34	.....
12,866...	2	.....	115,989...	1	.....
14,081...	7	1	117,424...	6	.....
15,033...	1	.....	117,731...	5	.....
15,479...	2	.....	128,164...	3	.....
17,607...	17	4	163,643...	12	.....
18,599...	3	.....	180,757...	2	1
19,345...	1	.....	229,364...	3	.....
20,900...	18	4	330,023...	2	.....
22,935...	3	.....			

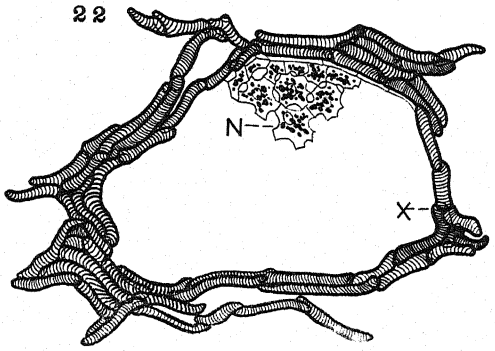
er than those of the apical meristem. The absolute increase in nuclear material estimated by comparison with the latter ranges from 86 to 232V.

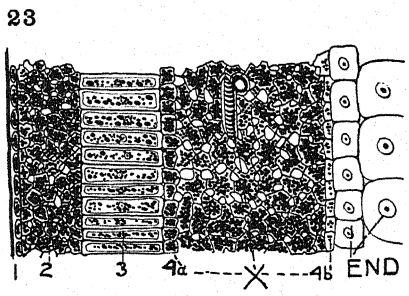
FRUIT AND SEED.—The pendent fruit, a capsule, is spherical to ellipsoidal in shape, densely spiny, and measures 6–8 cm. in length (fig. 21). The carpels range from two to six in number, and the placentation is axile (4). The seeds are ellipsoidal, about 1 cm. long, and vary from one to four per carpel. They are shed while the fruit is still green. The latter breaks open by ragged irregular tears around the beak and thereafter dries out and becomes brown and papery.

The surface of the young ovary is densely covered with trichomes and glandular hairs. Typical epidermal cells are approximately isodiametric, and stomata are frequent. The glandular hairs generally persist, more or less dried up, in the older fruits and may be identified even at the spine tips. The mesocarp of the young fruit is spongy and consists of chlorenchyma and parenchyma. Intercellular spaces appear early in the latter and indicate the future zones of tissue shrinkage and splitting which accompany locule development. The vascular network is highly complex. The cells of the endocarp are small and tabular and









FIGS. 20–23.—Fig. 20, *A–D*, varying outlines of nuclei; *E*, giant nucleus in differentiating vessel segment. Fig. 21, diagrammatic longisection of semimature green fruit with developing ovules, venation of carpel, and funiculus. Fig. 22, vascular network of nucellus. Fig. 23, semidiagrammatic transection of seedcoat of semimature seed: epidermis (*1*), parenchyma (*2*), palisade layer (*3*), nucellus with outer and inner limiting layers (*4a*, *4b*), and spiral vessels of vascular network.

generally adhere to the surface of the ripening seed.

The seed, when mature, is light brown or buff in color; the embryo is typically dicotyledonous. While unripe it is white,

TABLE 3

COMPARISON OF NUCLEAR DIAMETERS AND VOLUMES IN CLOSELY ADJACENT PARENCHYMA CELLS, LIVING AND NONLIVING, OF ECHINOCYSTIS MACROCARPA

Region of stem	No.	Dimension of cell (longi-section)	Nucleus		Diameter of nucleolus	Appearance of nucleus
			Diameter (μ)	Volume (cu. μ)		
C	1	475×114	38	28,670	5	S.T.*
	2	456×114	19	3,582	3	R.O.†
C	1	133× 66	24	7,890	5	S.T.
	2	98× 66	20	4,773	4	S.T.
	3	95× 66	11	771	3	R.O.
D	1	400× 72	32	17,607	5	S.T.
	2	440×120	17	2,935	4	R.O.

* Nucleus closely surrounded by chloroplasts and translucent.

† Chloroplasts removed from nuclear surface, nucleus opaque and either granular or recticular.

TABLE 4

RANGE OF CONTRACTION OF NUCLEI OF ECHINOCYSTIS MACROCARPA ON ADDITION OF ABSOLUTE ALCOHOL; VESSEL DIAMETER 100–200μ

Nuclear volume (cu. μ)	Volume after shrinkage (cu. μ)	Water loss (%)	Comparison with cambial volume (V=91 cu. μ)
28,670.....	7,890	72	86V
35,024.....	21,125	39	232V
96,762.....	16,385	82	185V

with a distinct ridge outlining the major ellipse. If the seed at this stage is removed from the capsule and exposed to the air, the tannin in the cells of the outer seedcoat immediately darkens to olive brown or gray, and the ridge stands out as a darker line. In such a semimature seedcoat four layers may be distinguished:

1. The epidermal cells, in general isodiametric, are thickened on the external walls and are already suberized. Starch and tannin are present throughout (fig. 23 *1*).
2. The underlying parenchymatous elements are loosely packed, isodiametric to oblong, with abundant starch and some tannin. In the mature seed the walls thicken and lignify, ending as a layer of fibrous sclerenchyma (fig. 23 *2*).
3. Palisade cells, 600–700 μ in length and about 60 μ in diameter, form the main strengthening layer of the mature seedcoat. At this early stage wall thickening is just beginning along the outer tangential and radial walls. Starch grains are rather numerous. Suberization, lignification, and further thickening of walls occur later (fig. 23 *3*).
4. The nucellus, bounded on the outer and inner surfaces by more or less tabular cells, consists of loose spongy tissue supported by a vascular network. The much-branched spongy cells are packed with starch, and intercellular spaces are definite. The lignified spiral elements of the vascular mesh are accompanied by a narrow line of phloem-parenchyma. The cells of the limiting layers are tabular and contain some starch (fig. 23 *4a*, *4b*, *X*; fig. 22).

The endosperm is clear and glassy and practically devoid of starch. In the larger central cells the nuclei are readily visible with a hand lens (fig. 23 *END*). The endosperm is partially surrounded by the cotyledons of the developing embryo. The cotyledonary cells are relatively small and packed with protein granules 1–2 μ in diameter. Addition of Millon's reagent results in the immediate deep-flesh coloration seen in the tuber paren-

chyma. A few small starch grains are detected in most cells on addition of IKI.

As the seed matures, the reserve starch disappears from integument and nucellus. The nucellus itself is crushed and more or less completely resorbed as the embryo replaces the endosperm. The seedcoat as a whole hardens with suberization and lignification of the epidermis and of the underlying parenchyma and palisade layers.

TABLE 5

NUCLEAR SIZE IN FRUIT AND SEED TISSUES OF ECHINOCYSTIS MACROCARPA

Region	Tissue	Cell dimensions (μ)	Diameter		Volume of nucleus (cu. μ)
			Nucleus (μ)	Nucleolus (μ)	
Fruit	Epidermal hairs	24×24	9	3	432
		114×24	11	3	771
	Chlorenchyma outer mesocarp	76×76	20	4	4,773
		171×85	19	3	3,582
	Parenchyma inner mesocarp	228×228	28	5	12,095
		322×190	38	5	28,670
Seed	Palisade cells	648×60	28	6	12,095
	Endosperm	228×228	41	7	33,327
		182×182	49	4	62,890
		436×228	57	3	96,762
		228×228	62	4	128,164

NUCLEAR SIZE IN FRUIT AND SEED.—The nuclei of the pericarp in general resemble those of the vegetative tissues in size range. Endosperm nuclei, at a certain stage of development, are conspicuously larger but do not equal the maximum volumes of the vessel segment nuclei. Table 5 gives typical figures.

B. CUCURBITA PEPO

The axis of *C. pepo*, reviewed fully by HAYWARD (4), is similar to that of *Echinocystis*. The spiral vessels of the primary bundles are followed by large pitted vessels right and left of the original xylem wedge. Later secondary vessels differentiate between the pitted laterals. The region of most active secondary growth, the seventeenth to nineteenth internodes, inclusive, in the specimens examined is the region most useful in the study of nuclear size (tables 6, 7, 8). The maximum volumes of nuclei in xylem and in parenchymatous cells, 4,958V and 1,156V, when reckoned in comparison with the nuclei of the meristem cells (V = 33 cu. μ), do not equal the largest

TABLE 6

NUMBER OF DIFFERENTIATING VESSELS, MAXIMUM VESSEL DIAMETERS, MAXIMUM NUCLEAR DIAMETERS, AND VOLUMES IN 40-CM. SHOOT OF CUCURBITA PEPO IN SUCCESSIVE INTERNODES

Region*	Internode number	Vessels			Nucleus	
		Number differentiating		Diameter of largest lignified vessel (μ)	Diameter (μ)	Volume (cu. μ)
		Inner bundles	Outer bundles			
B	14†...	34	39	66	28	12,095
	15...	25	25	83	51	70,521
	16...	12	3	142	38	28,670
C	17...	8	4	237	49	62,980
	18...	6	7	218	57	96,762
	19...	3	8	180	43	43,601
	20...	5	6	190	41	38,159
	21...	5	6	199	41	38,159
D	22...	0	2	171	47	55,992
	23...	3	4	218	38	28,670

* Apical region (A), one to thirteen internodes.
† Approximately.

nuclei seen in *Echinocystis*. It is possible that this is due to the fact that the *Cucurbita* seeds were sown rather late in summer, and growth was not so vigorous as in the early part of the season.

TABLE 7

RANGE OF NUCLEAR SIZE IN 215-CM. SHOOT OF CUCURBITA PEPO

Region	Cell		Nucleus		Nucleolus diameter (μ)	Scale of increase
	Type	Diameter (μ)	Diameter (μ)	Volume (cu. μ)		
A	Meristematic, dividing..	7– 8	4	33	2	V=33
B	Vessels.....	36– 85	13–51	1,200– 70,521	3–6	38–140V
C	Parenchyma.....	123–266	15–41	1,832– 38,159	3–6	55–1,156V
	Vessels.....	152–266	30–66	14,801–163,643	3–6	478–4,958V
D	Vessels.....	60–171	22–49	6,186– 62,980	3–6	187–1,908V

TABLE 8

VOLUME FREQUENCIES IN PARENCHYMA AND VESSELS OF CUCURBITA PEPO

Volume (cu. μ+)	Parenchyma	Vessels	Volume (cu. μ+)	Parenchyma	Vessels
1,000....	3	1	28,000....	.....	32
3,000....	3	4	33,000....	.....	13
5,000....	2	6	43,000....	.....	2
7,000....	2	10	55,000....	.....	8
9,000....	2	.....	62,000....	.....	14
11,000....	1	5	70,000....	.....	8
13,000....	1	12	79,000....	.....	1
17,000....	1	10	96,000....	.....	2
19,000....	.....	12	163,000....	.....	1
24,000....	.....	2			

Discussion

While apparently only one paper in the literature deals with nuclear size and vessel differentiation (9), considerable data has accumulated around the problem of nucleocytoplasmic ratios in general. Among other papers (11), the recent studies of the changing rates of growth in cells and in nuclei by SINNOTT and TROMBETTA (10) provide the most illuminating method of attack and graphic expression of the problem. Isolated facts observed in the present survey, if subjected to such statistical study and critical analysis, could be fitted into their place in the cycle of growth. For example: (*a*) The nuclei of very young differentiating vessel segments may measure approximately seven-eighths of the vessel diameter, indicating that the nucleus in the early stages of vessel growth increases much more rapidly than the protoplast as a whole. The point at which nuclear growth begins to slacken remains undetermined. (*b*) Nuclei in adjacent parenchymatous cells of exactly similar diameter and length may differ greatly in volume. This may be due to sectioning shock or to cell age. (*c*) In contrast to this, nuclei of like volume may appear in adjacent cells of equal diameter but of markedly different length and therefore volume. Since the size of nuclei from the apical meristem downward increases with increasing cell size (table 1), this uniformity requires explanation. Cell age may or may not be the reason.

The ratio of volume of cell/volume of nucleus is highly variable in *Echinocystis*, as in other plants (2, 10, 11). Calculations are valid only when based on detailed measurements. The following figures merely illustrate the range seen in

typical cells in various tissues: (*a*) meristem 15:1; (*b*) cortical parenchyma 12:1–113:1; (*c*) differentiating spiral vessel 18:1; (*d*) differentiating pitted vessels 34:1–152:1.

The volume frequencies in table 2 recall the serial figures of nuclear doubling of JACOBY (5) and MONSCHAU (6), V, 2V, 4V, 8V, which are refuted by WERMEL and PORTUGALOW (12). It is expected that a more complete statistical analysis of *Echinocystis* and also of *Ricinus* (9) would result in a uniform curve instead of a many-peaked graph. The theory of protomere division is not necessarily ruled out, but time intervals between individual divisions throughout the mass doubtless vary with internal and external milieu. As a result the stages in nuclear growth will probably present a gradual and continuous sequence.

More recent cytological work indicates that nuclear volume must be considered in relation to chromosome number (3). Whether the vessels and parenchymatous cells of *Echinocystis* and *Cucurbita* are actually polyploid is undetermined.

Nuclei as osmometers are discussed by CHURNEY in isolated *Arbacia* and other eggs (1). Certain tentative experiments were made with sections of *Echinocystis*, but the contents of the nuclei responsible for the increasing intake of water remain to be determined.

Summary

1. In *Echinocystis macrocarpa* the large differentiating vessels of the secondary xylem furnish excellent material for the study of giant nuclei.

2. The massive tuber is made up of storage parenchyma and a complex vascular network. Microscopically visible ergastic substances include abundant starch and a few small oil droplets. Immediate reaction with Millon's reagent indicates high protein. The core of the tuber is demarcated by amber color, lack of starch, and tissue shrinkage resulting in cleavage cracks.

3. Ergastic substances in the short-lived shoot are limited to rather abundant starch in the chlorenchyma of the outer cortex and in the sieve tubes and scattered starch grains and oil droplets in other tissues. High protein is indicated in sieve tubes and curiously also in the cell walls of collenchyma, pericycle, crushed phloem, and the xylem. Calcium oxalate is absent throughout practically the entire plant.

4. Structure and development of fruit and seed are outlined. Reaction is marked in the microscopically visible granular protein of the embryo cotyledons.

5. The differentiating vessels contain nuclei of relatively great size, the maximum 85 μ in diameter and 330,023 cu. μ in volume. This confirms the opinion expressed after the study of *Ricinus* that giant nuclei are of general occurrence in wide-vesseled plants.

6. The scale of nuclear growth is reckoned in comparison with the size of the meristem nuclei, 33 cu. μ = V. The cambial nuclei remain consistently small, 432 cu. μ = 13V, while the nuclei of hairs, collenchyma, and other vegetative tissues range from 4 to 187V. Endosperm nuclei measure 3,883V during early stages of growth.

7. Giant nuclei appear to be either in the resting or in the prophase condition. A surface network of chromatin is apparent. The nucleoli, one or more in number, are extremely variable in outline and contain minute, highly refractive droplets.

8. Shrinkage in volume, probably due to water loss, may result from sectioning. Nuclei in living parenchymatous cells

when first mounted are outlined by encircling chloroplasts, translucent and therefore practically invisible. Their diameter is approximately twice that of nuclei in exactly similar closely adjacent nonliving cells. (A nonliving cell is here defined as one in which no trace of protoplasmic rotation or circulation can be detected.)

9. Contraction in volume is induced by dehydration of fresh material with absolute alcohol. The volume of free water ranges from 39 to 82 per cent. Actual increase in nuclear material is reckoned by comparison of the contracted nuclei with the meristem nuclear volume, V = 33, and amounts to 86–232V.

10. The ratio of cell volume/nuclear volume in isolated typical cells from various tissues illustrates the variability of this figure (15:1 in meristem to 152:1 in vessels).

11. *Cucurbita pepo* is essentially similar to *Echinocystis* in the anatomy of the shoot. The nuclei in the developing vessels are somewhat smaller and reach a maximum of 66 μ and 163,643 cu. μ in diameter and volume.

12. Comparison of living material and prepared slides on both *Echinocystis* and *Cucurbita* indicates the necessity of the former in all studies pertaining to cell and nuclear growth.

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LITERATURE CITED

1. CHURNEY, L., Osmotic properties of the nucleus. Biol. Bull. **52**:52–67. 1942.
2. ENSIGN, M. R., Venation and senescence of polyembryonic citrus plants. Amer. Jour. Bot. 6:311–329. 1919.
3. GENTCHEFF, G., and GUSTAFSSON, Å., The double chromosome reproduction in *Spinacia* and its causes. Hereditas **25**:349–357; 371–386. 1939.
4. HAYWARD, H. E., Structure of economic plants. p. 584. New York. 1938.
5. JACOBY, W., Über das rhythmische Wachstum der Zellen durch Verdoppelung ihres Volumens. Arch. Entwicklungsmech. Organ. **106**:124–192. 1925.
6. MONSCHAU, M., Untersuchungen über das Kernwachstum bei Pflanzen. Protoplasma **9**: 536–575. 1930.
7. MUNZ, P. A., Manual of southern California botany. Claremont. 1935.
8. SCOTT, FLORA M., Differentiation of spiral vessels in *Ricinus communis*. BOT. GAZ. **99**:69–79. 1937.
9. ———, Size of nuclei in the shoot of *Ricinus communis*. BOT. GAZ. **101**:625–636. 1940.
10. SINNOTT, E. W., and TROMBETTA, V. V., Cytonuclear ratio in plant cells. Amer. Jour. Bot. **23**: 602–606. 1936.
11. TROMBETTA, V. V., The cytonuclear ratio. Bot. Rev. 8:317–336. 1942.
12. WERMEL, E. M., and PORTUGALOW, W. W., Studien über Zellengrösse und Zellenwachstum. Zeitschr. Zellforsch. **22**:185–194. 1934.

TRANSLOCATION OF THE FLORAL STIMULUS IN XANTHIUM[1]

ALICE P. WITHROW AND R. B. WITHROW

(WITH THREE FIGURES)

Introduction

HAMNER and BONNER (4) have shown that in a two-branched plant of *Xanthium*, if one branch (designated the donor branch) is induced to initiate flower buds by a short photoperiod treatment, the floral stimulus is translocated to the receptor branch kept on a long photoperiod and the receptor branch flowers. They have further shown that the stimulus will cross an inarch graft union under similar conditions with the same result. They also found that the receptor plant flowered if a membrane of lens paper was placed between the cut surfaces of the graft stems and concluded that the stimulus passed through a nonliving diffusion contact, and therefore that a definite substance was involved in causing flowering. On the other hand, MOSHKOV (5) concluded from his experiments with *Perilla* that tissue union between the scion and stock was necessary for the transfer of the floral stimulus.

Little work has been done to show through what tissues of the stem the floral stimulus travels from its point of origin to the induced bud. CAJLACHJAN (2) has reported that the stimulus in *Perilla* was translocated both up and down the stem in the tissues of the bark.

The present experiments were designed to study the translocation of the floral stimulus in *Xanthium pennsylvanicum* in relation to its movement through diffusion contacts and to the tissues involved in its translocation in the petiole and stem.

[1] Journal Paper no. 53 of the Purdue University Agricultural Experiment Station.

Investigation

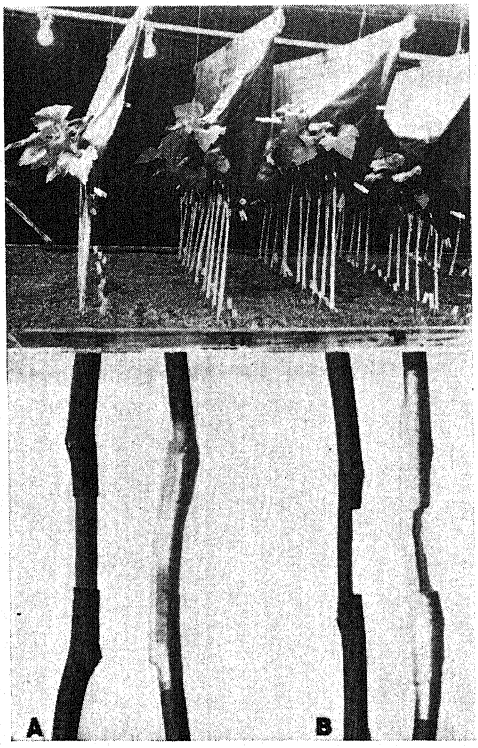
INARCH GRAFTS AND DIFFUSION CONTACTS

It appeared reasonable to assume that if the floral stimulus from a donor plant could cross a diffusion contact to a receptor plant, something of its nature could be found if various types of diffusion membranes were used. This would aid materially in selecting the most suitable solvent for extraction purposes.

Preliminary experiments using inarch graft contacts through various membranes were set up. Thin cellophane, thin rice paper (cigarette paper), and thin rice paper impregnated with various substances were used as membranes. The impregnating agents included lanolin, a polybutene–mineral oil mixture, and beeswax. It was found that the floral stimulus did not cross any of these contacts, and only those plants flowered where a direct graft was made and tissue union allowed to take place.

Following the preliminary experiments, it was decided to limit the types of membranes used and to incorporate experiments in which tissue union of direct type grafts was restricted. The following types of inarch contacts were made: (*a*) direct graft which was left undisturbed; (*b*) direct graft taken apart every 4 days, with the stem surfaces recut the first four times; (*c*) direct graft taken apart every 8 days, with new surfaces cut the first two times; (*d*) a lens-paper membrane interposed; (*e*) a thin rice-paper membrane interposed; (*f*) a lanolin-impregnated rice-paper membrane interposed.

In one case the donor plants of the pair were induced with short 10-hour photoperiods and allowed to come into flower before the contacts were made, at which time the short photoperiods were discontinued and the pairs of inarched plants placed on a 24-hour photoperiod



FIGS. 1, 2.—Fig. 1 (above), method used in curtaining donor plants. Fig. 2 (below), stem of donor branch: *A*, from which ring of bark has been removed, before and after taping; *B*, from which half-section has been removed, before and after taping.

(continuous irradiation) of solar irradiation supplemented with 20 footcandles of incandescent lamp irradiation. In these and subsequent experiments the receptor plants were defoliated to two well-developed leaves at the time of grafting and the burs were removed from the donor plants at intervals throughout the test period. This experiment was conducted during April and May.

In the second case induction of the donor was begun at the time the grafts were made, and the donor plant was curtained with heavy rubberized black cloth throughout the experimental period so as to receive 10 hours of solar radiant energy daily, while the receptor plants were subjected to the 24-hour photoperiod just indicated. This work was done during June and July. Figure 1 shows the grafted plants with curtains in place. All contacts were of the inarch type, about 20 cm. in length, and were securely bound with Parafilm tape. Rubber bands which had been cut into single lengths were wrapped around the outside of the Parafilm tape. This procedure kept the two stems in close contact but allowed adequate opportunity for stem growth without girdling.

The plants were grown in pots in sub-irrigation gravel culture beds with a complete nutrient solution. At the end of the experimental period of 50 days, freehand transections of the contacts were made in most cases to determine whether tissue adhesion or actual tissue union obtained. In all cases the grafts and contacts were subsequently taken apart and the condition of the membranes and state of tissue union noted. Dissection under a low-power binocular microscope was made for flower buds in all the experiments.

Table 1 summarizes the results. No receptor plants flowered except those which had formed tissue union with the donor plants. The flowering was restricted largely to those plants with direct undisturbed grafts and those with lens-paper membranes. Tissue union seldom failed to occur under both conditions, although frequently the areas of union were very small when lens paper was interposed between the cut surfaces.

Of thirty-three receptor plants where the rice-paper membranes were used, only one flowered, and it was found that the paper had disintegrated early enough to allow tissue union to take place. A similar situation existed where one receptor plant with a lanolin–rice-paper membrane flowered.

When the donor plants were induced previous to grafting and the short photoperiods over the donor discontinued at the time of grafting, less than half the plants in the treatments with direct graft and lens-paper membrane flowered, even though tissue union occurred. When the induction was begun at the time of grafting, 90 per cent or more of the plants flowered. When the direct contact was disturbed every 4 days, no flowering occurred although tissue adhesion had taken place at the end of 4 days. When the direct grafts were taken apart every 8 days, some of the plants eventually flowered, although the flowering was delayed as compared with undisturbed direct grafts. With freehand transections of test plants, tissue union was observed in about 60 per cent of them when the grafts were left in direct contact for 8 days. It is entirely possible that union occurred on the remainder of the plants at some point on the graft not observed with the microscope. Strong adhesion occurred in all cases.

TABLE 1

FLOWERING RESPONSE OF RECEPTOR PLANTS OF XANTHIUM

Treatment	I. Donors induced previous to grafting	II. Donors induced after grafting
	Receptors with flower buds 39 days after grafting* (%)	Receptors with flower buds 28 days after grafting* (%)
Direct graft	39	93
Lens-paper membrane	39	100
Rice-paper membrane	0	7†
Lanolin-impregnated rice-paper membrane	0	7†
Direct contact disturbed every 4 days	0	0
Direct contact disturbed every 8 days	0	20

* No change in flowering response at 50 days; 18 pairs of plants per treatment in I and 15 pairs in II.

† Tissue union between the donor and receptor plants occurred where flowering took place.

The plants with the lens-paper membranes developed burs more rapidly than did those with direct grafts. This was observed in both experiments.

TRANSLOCATION ACROSS DEAD PETIOLE TISSUE

Since it appeared that tissue union was necessary for transfer of the floral stimulus from one plant to another, it was thought desirable to determine whether the stimulus could be translocated from the induced leaf of a plant across a small length of dead petiole tissue.

The plants, growing in subirrigation pot culture with complete nutrient solution, were defoliated to two young mature leaves and the younger developing leaves. One mature leaf was left intact and uninduced; the second received the following treatments, 25 plants per treatment: (*a*) uninduced on long photoperiod; (*b*) induced on short photoperiod with intact petiole; and (*c*) induced on short photoperiod with petiole section approximately 1 cm. in length killed by steaming for 15 seconds.

The petiole to be treated with steam was incased in two pieces of slit rubber tubing which were separated so as to expose 1 cm. of the petiole to the steam. The petiole was then clamped between two blocks of wood for further protection. Steam was applied by means of a

small glass jet inserted into a 1-cm. hole drilled in the wood blocks. Immediately after steaming, the petiole was bound

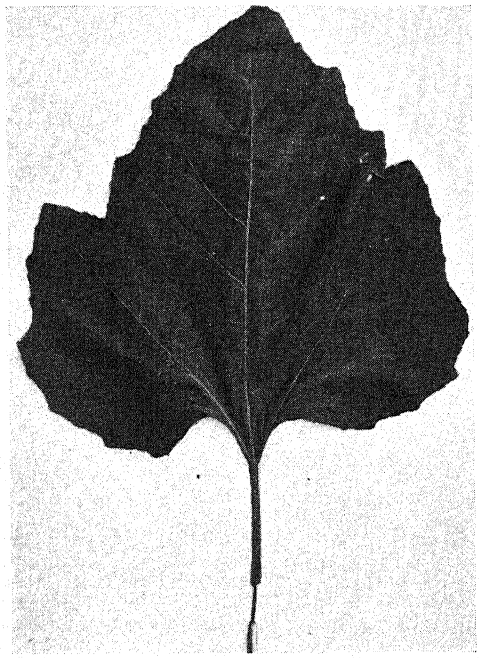


FIG. 3.—Leaf 24 hours after petiole section was killed by steaming for 15 seconds.

TABLE 2

EFFECT OF KILLING PETIOLE SECTION OF INDUCED LEAF ON FLOWERING RESPONSE OF XANTHIUM

Treatment	Plants with flower buds at 31 days* (%)
Short photoperiod, petiole intact....	84
Short photoperiod, petiole steamed 15 seconds.....	0
Long photoperiod, petiole intact....	0

* No change in flowering response at 40 days; 25 plants per treatment.

with thin wood splints and Parafilm tape to keep it in position. Figure 3 shows a leaf on the morning following the steaming treatment. The steamed petiole sections were greatly shrunken and completely brown at this time.

All the plants were kept on a 24-hour photoperiod of solar radiant energy supplemented by 20 footcandles of incandescent lamp irradiation. The leaves to be induced were placed in heavy black-paper envelopes each day at the end of 10 hours of solar irradiation. These leaves were removed from all the plants after 4 days of induction. At this time all the leaves appeared turgid and healthy, including those with steamed petioles. Preliminary experiments had shown that considerable wilting of the leaves with steamed petioles took place 5 days after treatment. The results are given in table 2. Only those plants flowered which had induced leaves with intact petioles.

TRANSLOCATION OF FLORAL STIMULUS DOWN STEM

Since it appeared from the previous experiments that the floral stimulus failed to diffuse across a nonliving membrane and that it was not translocated down through a section of dead petiole tissue whose xylem vessels were functional, it was thought likely that the stimulus was translocated within the plant through the living cells of the stem. An experiment was conducted to determine whether the downward movement of the stimulus was restricted to the bark.

The apical bud was pinched from a group of plants and two equal-sized branches allowed to develop. When these were large and well developed, the plants were divided into three groups. In all cases, one (donor) branch of each plant was curtained with the black cloth so as to receive a 10-hour photoperiod. The second (receptor) branch received a 24-hour photoperiod of solar radiant energy supplemented by 20 footcandles of incandescent lamp irradiation. In one

group the plants were left intact as controls; in the second group a ring of bark 4 cm. long, completely girdling the stem, was removed from the donor branch about 20 cm. above the point of branching. In the third group a half-section of the stem about 4 cm. in length was removed from the donor branch at a similar point to check the possibility of the influence of tissue injury or reduction in amount of stem tissue on the translocation of the floral stimulus. On fifteen plants in each treatment the donor branch was removed at the end of 12 days' induction and on twenty plants at the end of 8 days. These two experiments were conducted at different times. Microscopic examination of freehand stem sections of plants grown in preliminary experiments showed that considerable regeneration of tissue occurred 8–16 days after tissue removal, depending upon growth conditions, and that formation of vascular elements occurred within a slightly longer period. Microscopic examination of freehand transections of the girdled stem portion was made at intervals on test plants during the experimental period, and transections of the center area of all the girdled stem portions were examined at the time the donor was removed. In this area no regeneration of tissue was found on many plants, while on others there were occasional small groups of newly formed parenchyma cells present external to the xylem. Whether these groups were continuous throughout the length of the girdle was not determined.

Figure 2 shows the stems after tissue removal before and after taping. Large branches were most suitable, since their bark could be removed without scraping. In small young plants the bark does not peel off readily.

The lower leaves of both branches were removed in all cases at the beginning of the induction period, and the induced leaves on the donor branch were above the point of tissue removal. The plants were grown in subirrigation gravel culture with complete nutrient solution.

The results are given in table 3. The receptor branches showed macroscopic floral buds as early as 15 days after induction of the donor branch was begun.

TABLE 3

EFFECT OF REMOVAL OF STEM TISSUES OF DONOR BRANCH ON FLOWERING OF RECEPTOR BRANCH IN TWO-BRANCHED PLANTS OF XANTHIUM

Treatment	I. Receptor branches with flower buds at 25 days* (%)	II. Receptor branches with flower buds at 32 days* (%)
Donor branch intact....	100	.....
Donor branch with half-section removed.....	73	80
Donor branch with bark removed.....	0	5

* No change in flowering response at 45 days; 15 plants per treatment in I with donor branch removed after 12 days' induction and 20 plants per treatment in II with donor branch removed after 8 days' induction. No intact plants included in II.

However, the number of days given in the table is that after which no change occurred in the number of flowering receptor branches.

TRANSLOCATION UP STEM

Two-branched plants were divided into three groups, one branch serving as donor and given short photoperiods and the second serving as receptor and kept on long photoperiods. In these experiments it was the donor branch which was left intact. In group I the receptor branches were also left intact. In group II a complete section of bark 4 cm. long, entirely girdling the stem, was removed from the receptor branch 20 cm. above the point of branching. In group III a half-section 4 cm. in length was removed

from the stem of the receptor branch 20 cm. above the point of branching. Immediately after tissue removal, the treated stem portions were bound with Parafilm tape and supported with thin wood splints taped on to the stem. Just above the point of tissue removal the stem was wrapped with moistened sphagnum moss to facilitate the development of roots. This was done both where bark and half-stem sections were removed. In preliminary experiments those portions of the plant above the point of bark removal developed severe mineral deficiencies in 2–3 weeks, since the translocation of mineral elements was restricted. Such receptor branches could not be kept alive long enough to secure complete flowering data unless provision was made for a supply of mineral nutrients through a new root system developed above the girdle.

TABLE 4

EFFECT OF REMOVAL OF STEM TISSUES OF RECEPTOR BRANCH ON ITS FLOWERING IN TWO-BRANCHED PLANTS OF XANTHIUM

Treatment	Receptor branches with flower buds at 23 days* (%)
Receptor branch intact	100
Receptor branch with half-section removed	80
Receptor branch with bark removed	0

* No change in flowering response at 45 days; 20 plants per treatment with receptor branch removed after 14 days.

Immediately after tissue removal the donor branches were given short photoperiod treatment, which was continued for 14 days. At the end of this time the receptor branches, which now had well-developed roots, were removed from the plant immediately above the point of tissue removal and planted in subirrigation gravel culture with complete nutrient solution.

The results are given in table 4. The plants which were left intact all flowered, and 80 per cent of those with the half-section removed from the receptor branch flowered. Those with a complete ring of bark removed from the receptor branch failed to form floral primordia.

Discussion

The failure of the floral stimulus to cross the membranes used does not mean that there was no diffusion through them, but it does mean that the diffusion was not in sufficient quantity to cause flowering. Callus formation on the cut surfaces also may have decreased the opportunity for passage. These limitations, however, do not apply to the series disturbed every 4 days. Tissue adhesion occurred by the end of each 4-day period for the first 16 days, although no signs of union were observed when microscopic examination was made on test 4-day grafts run simultaneously. That the flower-forming stimulus failed to cross this tissue-to-tissue contact indicated that, under the conditions of the experiments, it was necessary for union to take place before translocation from the donor to the receptor plant could occur. Even with direct undisturbed grafts, translocation did not always take place. It hardly could be expected that the stimulus would cross a nonliving membrane if it failed to cross a tissue-to-tissue contact. These results are in harmony with those of MOSHKOV (5), who failed to secure transfer of the floral stimulus from the donor to the receptor in *Perilla* unless the scion had been kept on the stock more than 10 days. Tissue union occurred by 9–12 days, and MOSHKOV concluded that such union was necessary before the transfer of the floral stimulus could occur.

The floral stimulus in *Xanthium* moves both up and down the stem, as evidenced

by the fact that flowering takes place both above and below the point of induction. Since the translocation gradient through the xylem of plants is usually upward, it appears unlikely that any downward movement of the floral stimulus would occur in this tissue. Further, HAMNER and BONNER (4) have shown that placing plants in a saturated atmosphere did not affect the flowering in *Xanthium*, indicating that it was not dependent upon the transpiration stream.

In the present experiments the failure of the stimulus to cross the dead petiole section whose xylem vessels were functional all during the induction period, as evidenced by the turgid condition of the leaf, made it apparent that the stimulus did not move down the petiole in nonliving cells or through functional xylem. This is in harmony with results of other workers. When BORTHWICK and PARKER (1) maintained low temperatures around a section of the petiole of a Biloxi soybean leaf during induction, or around the stem immediately below the apical bud, the plant failed to form floral primordia. This is indicative that living cells were involved in the translocation of the floral stimulus from the induced plant portion to the bud and that the low temperature inhibited the activities of the living cells, thus preventing the transport of the floral stimulus. CAJLACHJAN (3) used low temperatures and narcotic applications on stem portions of *Perilla* and was able to prevent translocation of the floral stimulus from the induced plant portion. He concluded that living cells were involved in the translocation of the floral stimulus.

The experiments in which the bark was removed, leaving intact and functional xylem and pith, show that the floral stimulus does not move either up or down the stem in the xylem or pith to any appreciable extent but moves chiefly in the bark. It would appear likely that the floral stimulus is translocated in the phloem, since this is the tissue primarily concerned with translocation. In some cases, plants with half-sections of the stem removed failed to flower, probably because sufficient stimulus to cause flowering was not translocated before removal of the donor branch.

It generally has been assumed that the effects of girdling are due to the accumulation of carbohydrates above the girdled portion of the stem. Since girdling also appears to block the movement of the floral stimulus, it may be that the effects of girdling on flowering, as reported by ROBERTS and STRUCKMEYER (6) and others, are due to interference with the movement of factors controlling phasic development as well as to interference with the movement of carbohydrates.

Summary

1. The floral stimulus in *Xanthium pennsylvanicum* crossed an inarch contact between an induced donor plant and a receptor plant only when tissue union was established and direct tissue contact was uninterrupted for more than 4 days.

2. The floral stimulus failed to be translocated downward from an induced leaf through dead petiole tissue or through functional xylem cells.

3. The floral stimulus failed to be translocated from an induced donor branch of a two-branched plant to a receptor branch when the bark was removed from the donor branch below the induced leaves or from the receptor branch immediately above the point of branching.

4. The floral stimulus is translocated chiefly in the bark, both up and down the stem.

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LITERATURE CITED

1. BORTHWICK, H. A., PARKER, M. W., and HEINZE, P. H., Influence of localized low temperature on Biloxi soybean during photoperiodic induction. BOT. GAZ. 102:792–800. 1941.
2. CAJLACHJAN, M. CH., Motion of blossom hormone in girdled and grafted plants. Compt. Rend. (Doklady) Acad. Sci. URSS 18:607–612. 1938.
3. ———, Translocation of flowering hormones in the plant as affected by temperature and narcotics. Compt. Rend. (Doklady) Acad. Sci. URSS 31:949–952. 1941.
4. HAMNER, K. C., and BONNER, J., Photoperiodism in relation to hormones as factors in floral initiation and development. BOT. GAZ. 100:388–431. 1938.
5. MOSHKOV, B. S., Transfer of photoperiodic reaction from leaves to growing points. Compt. Rend. (Doklady) Acad. Sci. URSS 24:489–491. 1939.
6. ROBERTS, R. H., and STRUCKMEYER, B. ESTHER, The effects of temperature and other environmental factors upon the photoperiodic responses of some of the higher plants. Jour. Agr. Res. 56: 633–678. 1938.

NATURE AND RATE OF DEVELOPMENT OF ROOT SYSTEM OF CONVOLVULUS ARVENSIS[1]

JOHN C. FRAZIER

(WITH NINE FIGURES)

Introduction

In view of the seriousness of the weed situation and the desirability of a greater knowledge of the characteristics and habits of individual kinds of weeds, in order to combat them most efficiently, a study of field bindweed, *Convolvulus arvensis* L., was undertaken. According to HEGI (3) this plant is a native of Eurasia and is now extensively distributed, especially in the temperate zone of Eurasia and North America. It is especially troublesome in regions of limited rainfall because it competes so successfully with cultivated plants for soil moisture. It is perhaps the most noxious weed of the central United States.

GUNTHER (2) states that *C. arvensis* was described by DIOSCORIDES in his Herbal, which was compiled in the first century. According to SACHS (11), HIERONYMUS BOCK described this plant in his Herbal of the sixteenth century.

MALPIGHI was the first (1681) to report the development of adventitious buds on the roots of herbaceous plants, a process he described in some detail for an unnamed species of *Convolvulus*, which IRMISCH (5) believed was *C. arvensis*. Details regarding germination, general nature, rate of development, and regeneration of *C. arvensis* and *C. sepium* were given by IRMISCH, who lamented that in the older and newer descriptions of that day the subterranean parts either received little consideration or were not studied with exactness. He stated that the misconception that herbaceous roots could not bear adventitious buds would not have prevailed so long had other workers not disregarded MALPIGHI's work.

LUND and ROSTRUP (9) studied the root system of Canada thistle, *Cirsium arvense*. They described a type of development which involves lateral roots that grow horizontally for a greater or lesser distance and then turn downward into the soil, with new lateral roots arising at the bend to continue the plant horizontally.

ROGERS, DURRELL, and DANIELS (10) established the following facts regarding *Convolvulus arvensis:* (*a*) that the horizontal roots extend some 2–4 feet and then grow down into the subsoil to the water table, unless the latter is too deep; (*b*) that at the point of turning down a new horizontal root arises and continues in the same general direction; and (*c*) that buds on the root at the bend develop shoot growth.

KENNEDY and CRAFTS (6) called attention to the influence of external factors on the growth habit, especially the relation of favorable soil conditions to the development of permanent lateral roots. They concluded that the marked ability of this plant to produce buds in great numbers is correlated with the fact that the root has many live cells in close proximity to an extensive supply of stored food.

The observations of BAKKE (1),

[1] Contribution no. 439, Department of Botany, Kansas Agricultural Experiment Station.

HITCHCOCK and CLOTHIER (4), KIESSELBACH *et al.* (7), and KILTZ (8) on the nature of the vertical and horizontal portions of this root system agree with the findings of this study.

Observations

SOIL DATA.—All plants[2] considered in this paper were taken from soil, the nature of which is described in the following profile description supplied by the late Dr. W. H. METZGER, of Kansas State College:

Depth 0–15 inches.—A dark brown, granular silt loam; structure not strongly developed; organic matter content medium; loose and friable.

Depth 15–21 inches.—A dark brown silt loam but slightly lighter in color than preceding layer; structure particles larger than in layer above and tending to group into columns.

Depth 21–29 inches.—A brown silty clay loam, more compact than preceding layers; low in organic matter; probably the least permeable layer of the profile but should permit relatively free development of roots.

Depth 39–78 inches.—A brown to grayish brown silt loam containing a network of lime streaks; fragmental structure weakly developed; slight tendency toward columns; more friable than layer above.

Depth 78–144 inches.—Brown silt loam with less lime than layer above, except in lowest 6 inches; very weakly columnar.

Depth 12–18 feet.—Grayish brown silt loam, high in lime content; very pervious.

Depth 18–21 feet.—Brown and slightly finer textured than layer above, probably just barely within silty clay loam class; pervious and containing less lime than at 12–18 feet.

Depth 21–23 feet.—Very much like that at 18–21 feet, except that grayish brown and occasionally very narrow bands of sand are intermingled with the finer textured material; very pervious.

Depth 23–24 feet.—Mottled gray and brown, the brown occurring in streaks and having a rusty appearance, indicating iron; sand layers, somewhat wider than at 21–23 feet, intermingle with silt loam; very pervious and mellow.

No layer of this profile appears to be of such nature as to impede seriously the development of the roots of deep-rooting plants.

METEOROLOGICAL DATA.—The monthly and annual temperatures and precipitation for the first 10 months of 1937 compared with the 81-year average (1858–1938) of these two factors are given in table 1.

TABLE 1

MONTHLY AND ANNUAL TEMPERATURES (IN °F.) AND PRECIPITATION (IN INCHES) AT MANHATTAN, KANSAS, 1937

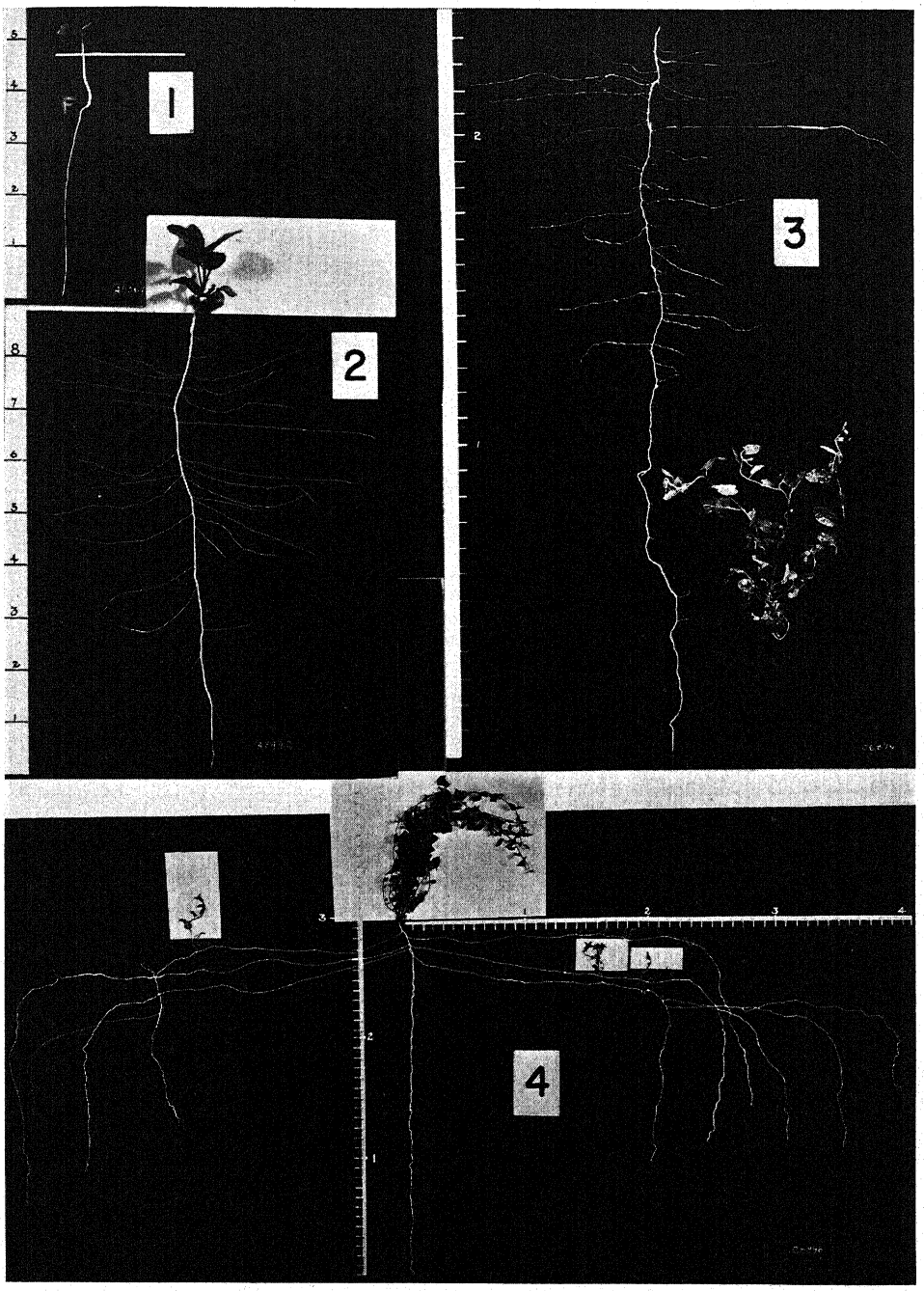
Month	Temperature		Precipitation	
	Mean*	81-year average (1858–1938)†	Total*	81-year average (1858–1938)†
Jan.	21.6	27.3	1.76	0.75
Feb.	30.6	31.5	0.91	1.14
Mar.	39.6	42.5	1.75	1.47
Apr.	54.8	54.6	0.54	2.66
May	67.2	64.7	2.42	4.34
June	76.0	74.5	3.39	4.47
July	82.9	79.8	4.39	4.32
Aug.	84.6	78.2	2.05	3.72
Sept.	72.6	69.9	1.52	3.49
Oct.	56.6	57.1	2.15	2.22
Annual mean or total	54.8	54.48	21.81	30.94

* Meteorological data obtained from U.S. Weather Bureau, Kansas Section.

† Computed by Dr. A. B. CARDWELL, of Kansas State College.

The year 1936 immediately preceding this investigation was one of temperature extremes. The 30-day period ending February 21, 1936, was the coldest on record. That summer was the second hottest on record. There were 60 days when the maximum temperature reached 100° F. or above, during June, July, August, and September. The average number of days per year at this temperature for the last 50 years (1889–1938) is 15. The 1936 rainfall was deficient 6.4 inches as compared with the 81-year average.

[2] The term plant as used in this paper refers to all the growth from a single seed. The term shoot is applied to the individual leafy stems, commonly called plants in control studies.



FIGS. 1–4.—Stages in development of plants of field bindweed: Fig. 1, seedling 1 week after emergence; *F*, root-stem transition zone. Fig. 2, young plant 4 weeks after emergence (not all vertical root obtained). Fig. 3, 9 weeks after emergence (shoots and roots separately). Fig. 4, 11 weeks after emergence; lateral roots arranged to show development of rhizomes, shoots, and permanent lateral roots of second order; hence, some laterals appear to be above the shoots of others. Figs. 1, 2, scale in inches; figs. 3, 4, scale in feet and inches.

January, 1937, was the fifth coldest January in 50 years. The following summer was hot, with temperatures of 100° F. or higher prevailing for 38 days. January, 1937, had more than 1 inch of precipitation above the 81-year average. March and July were the only other months that exceeded this long-time average. The average monthly precipitation for the first 10 months of 1937 was 2.09 inches as compared with the monthly average of 2.86 inches for these months during this long-time period.

Methods

A small plot of land free of both bindweed and sodium chlorate was used. The soil profile, already given, showed a rather typical Geary silt loam, representative of the wind-deposited soil of the upland of this area. The plot had been cultivated but was undisturbed otherwise.

Plantings were made in late October, 1936. Fifteen seeds were placed 1 inch below the surface at each of twenty-five points, which were so spaced that eight of the plantings were 18 feet distant from any other and the remainder were separated one from another by 8–9 feet.

Emergence of seedlings began April 4, 1937, and on April 9 at least one seedling emerged at twenty of the twenty-five points. All other seedlings were removed, as were those emerging after that date. All plants shown in the accompanying illustrations emerged on April 9. The area was kept free of other vegetation, so the only competition for water and nutrients, if any existed, was among the bindweed plants.

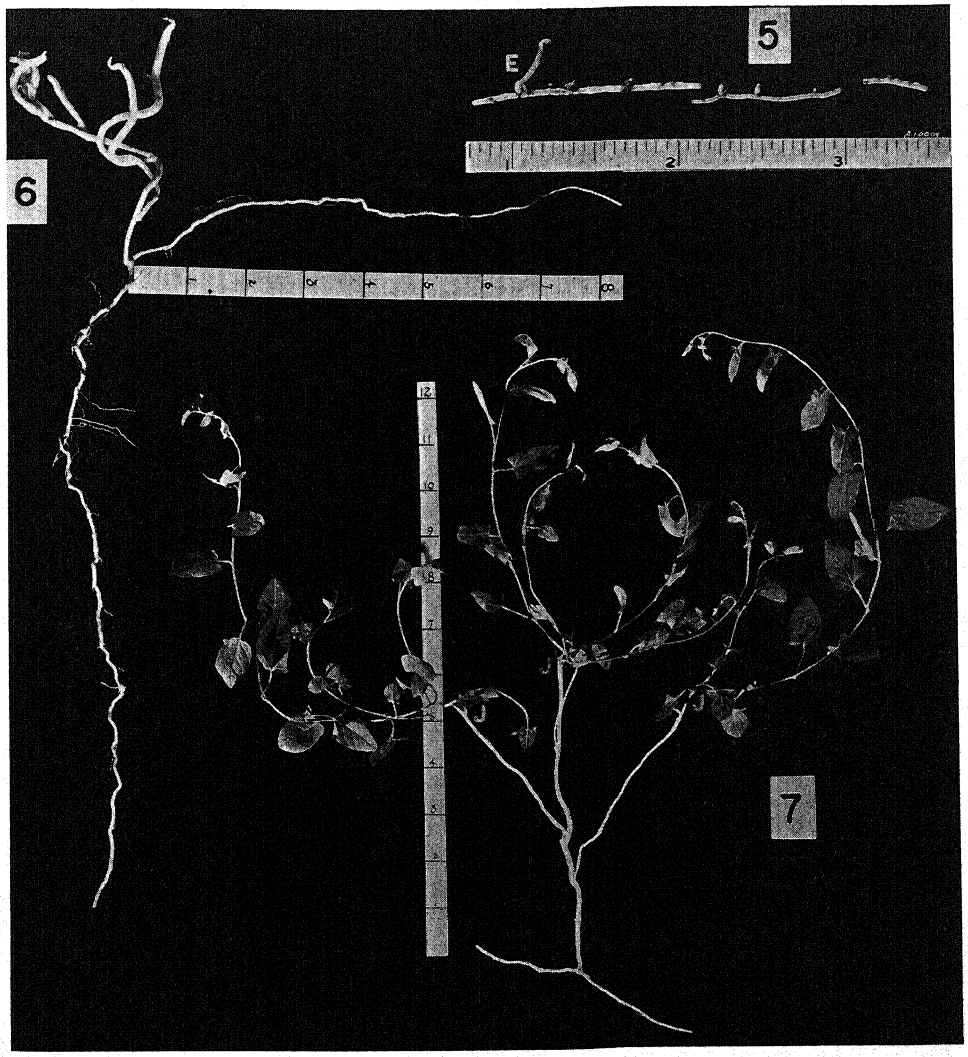
The root systems were excavated by a modification of the trenching method developed by WEAVER (12), which involved the use of a hand-pick and an ice-pick to expose and free the underground parts, after suitable trenches had been made.

Effort was made to keep the root system as one organic entity, hence, when it was broken, the two ends were immediately tied together. Measurements of the plant parts were made and recorded, and the relationship of parts noted as the root system was excavated, so that the plant as illustrated occupied the same relative relationship, one part to another, as it did in the soil (except that figures 2–8 have all lateral roots arranged in one plane). While as many of the finer roots were obtained as possible, it is not contended that many were secured, except for the plant shown in figure 2, in which case a fine jet of water was employed to free the roots from the soil.

Results

Plants were excavated at weekly intervals for the first 11 weeks, and also the thirteenth and the twenty-ninth weeks after emergence. The plant taken the thirteenth week after emergence (fig. 8) showed its gross morphological nature particularly well, especially the root system. A taproot rapidly penetrated directly down from the germinating seed. It was a primary root in order of development and vertical in position, and hence is designated the primary vertical root (fig. 8*P*). Many branch roots arose throughout the length of this taproot, most of them small feeding roots. A few, probably those more favorably located, grew extensively and became permanent parts of the root system. They are designated permanent lateral roots (fig. 8*L*). They grew horizontally 15–30 inches and then turned down in a broad sweeping bend and were from that point vertical taproots, designated secondary vertical roots (fig. 8*S*). The horizontal portions of the lateral-vertical roots of any order, which are usually smallest in diameter at their origin, were nearly always in the surface foot, more commonly in the upper 4–6 inches of the soil.

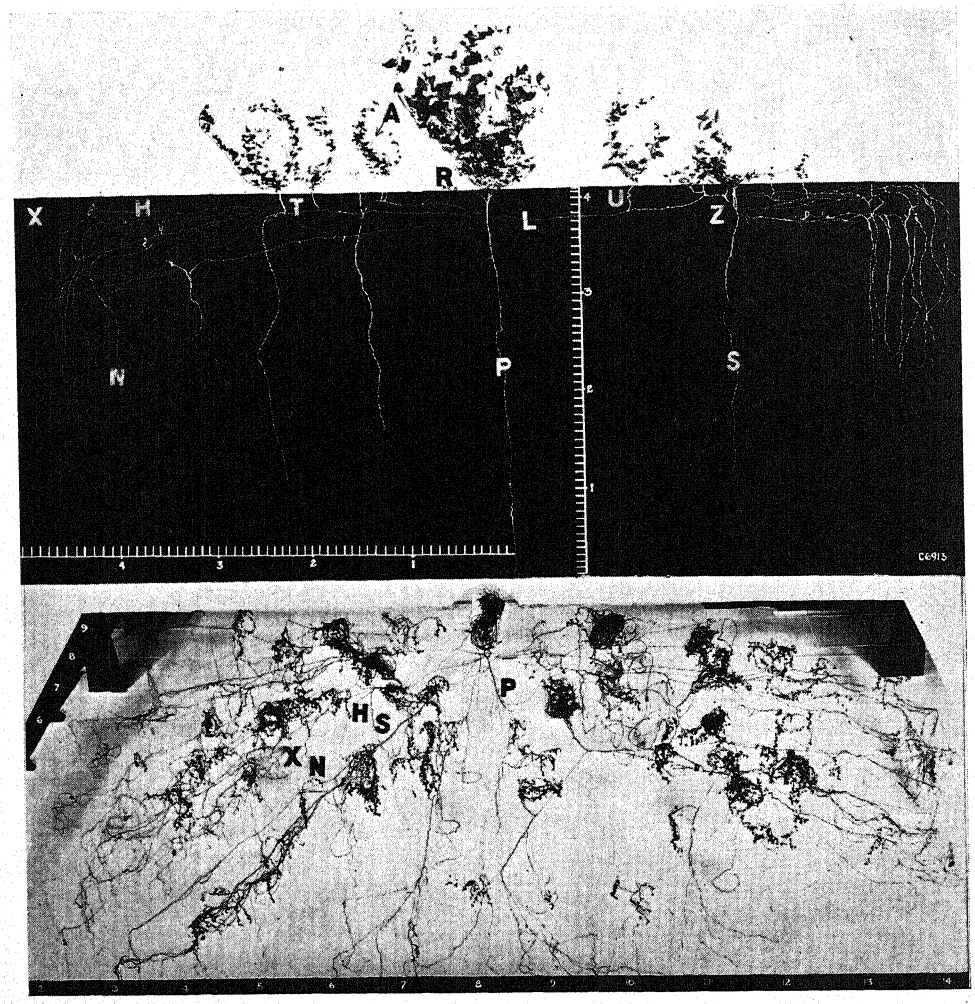
These lateral-vertical roots of the first order gave rise to branch roots over any part of their horizontal or vertical extent. Most of them remained as small feeding roots. However, at or just below the bend where the permanent lateral roots became secondary vertical roots, permanent laterals of the second order formed (fig. 8*H*). They extended horizontally 10–20 inches from their point of origin, the general direction of growth being away from the primary vertical root, and turned down to form secondary vertical roots of the second order (fig. 8*N*). This development was repeated many times during the growing season to give rise to



FIGS. 5–7.—Fig. 5, permanent lateral root showing development of root-borne stem buds, which if borne underground give rise to rhizomes (*E*). Fig. 6, heavy rhizome arising at juncture of permanent lateral and secondary vertical roots, showing scale leaves at nodes and branching. Fig. 7, short section of permanent lateral root bearing branched vertical rhizome, upper parts of which are leafy stems above ground line (*J*). Scales in inches.

lateral-verticals of the third, fourth, and even higher orders. This is suggested at *X* of figure 8 and is shown to advantage in figure 9. It is by such series of permanent lateral roots that horizontal spreading of the plant is accomplished.

If more than one permanent lateral root arose from the place of origin of such roots of the second or higher order (which is on or just below the bend separating the permanent lateral root of any order from its vertical phase), they radiated somewhat from the point of origin in their growth; but in general all maintained growth away from the primary vertical root. This resulted in the plant's



FIGS. 8, 9.—Fig. 8 (above), complete plant 13 weeks after emergence. *P*, primary vertical root; *L*, permanent lateral root of first order; *S*, secondary vertical root of first order; *H*, permanent lateral root of second order; *N*, secondary vertical root of second order; *X*, permanent lateral root of third order; *T* and *Z*, heavy rhizome and shoot development on or near juncture of permanent lateral and secondary vertical roots; *A*, the one flower on this plant; *U*, rhizome arising from lateral root; *R*, shoot from rhizome borne in primary vertical root. Scale in feet and inches. Fig. 9 (below), approximately half a plant 29 weeks after emergence. The growth, supported by strings placed at foot intervals on a 10 × 16 foot frame, is at such an angle that some roots may appear to be above some of the shoots. Scale in feet.

occupying a higher proportion of the ever-increasing area than it would have otherwise. This area was somewhat circular in shape.

All shoot development, except that from the plumule, was derived from of the fact that the original growth from the plumule was augmented by the development of axes from its cotyledonary and other nodes.

The location of heaviest shoot development from root-borne stem buds

TABLE 2

RATE AND NATURE OF DEVELOPMENT OF PLANTS OF BINDWEED AT INDICATED INTERVALS AFTER SEEDLING EMERGENCE; MANHATTAN, KANSAS, 1937

Weeks after emergence	Maximum vertical penetration (inches)	Maximum radial spread (inches)	Plant condition
1	4¾	.....	Cotyledons distinctly shaped and incised on adaxial side (fig. 1)
2	6¼	2	First two true leaves
3	9	3	Additional true leaves; cotyledons still present
4	9	3¾	Primary stem axis beginning to elongate (fig. 2)
5	11	4¼	Cotyledonary buds developing shoots
6	12¼	4½	Other axillary buds becoming active
7	13¼	7	Primary stem axis markedly elongated and nutating for support; cotyledons withering
8	18	7½	All secondary stem axes elongating rapidly; cotyledons lost
9	28½	10½	Primary and secondary stem axes 7–8 inches long
10	34	21½	First root-borne stem bud on lateral root 13 inches from primary vertical root
11	36	42	Root-borne stem buds (fig. 4) have developed rhizomes shown in detail in figs. 5 and 6. Three of the 52 rhizomes have leafy shoots (fig. 7)
13	49½	63	Flower in bloom (fig. 8*A*); permanent lateral roots of first (*L*), second (*H*), third (*X*) orders. Typical bend shown at *T* and *Z*, with rhizomes and their shoots and lateral roots of second order (*H*). Sixty-three rhizomes beyond bud stage, many of them on laterals of second order (to right of *Z*). Thirteen rhizomes bore leafy shoots, two on permanent lateral roots of second order. Shoot borne by rhizome (*R*) on primary vertical root
29	46	10 ft., 4	Approximately half a plant (fig. 9). Complete plant had twenty permanent lateral roots of first order, ten of which are shown with almost all their growth and with place relationship of parts essentially as they were in the soil*
120†	23 ft., 1½	At least 16–17 ft.	Secondary vertical roots extended 16–18 feet, with one traced to more than 23 feet‡

* No other plant studied had more than twelve permanent lateral roots of first order.

† 1939.

‡ No primary vertical root was found deeper than 14 feet 8 inches.

root-borne stem buds. These developed on any part of the permanent root system (that is, the two types of vertical roots and the permanent lateral roots). The shoot growth shown directly above the primary vertical root in figure 8 is made up predominantly of stems originating from root-borne buds formed on the primary vertical root in the upper inch or two of soil. This occurred in spite

was at the bend separating the lateral root of any order from its vertical phase (fig. 8*T*, *Z*). This was the site of heavy shoot development in the older plant shown in figure 9. Less commonly, shoots were found along the horizontal portion of permanent lateral roots (fig. 8*U*).

Regardless of where these root-borne buds formed, if they developed they gave

rise to rhizomes, unless the buds were borne at or above the ground line, in which case they gave rise to leafy shoots. Figure 5 shows such a series (right to left) by which these buds develop into rhizomes (*E*). Figure 6 shows a well-developed rhizome which has branched, but no branch of which has reached the surface, with the location of its nodes indicated by the scale leaves. In the axils of these leaves are buds which, if they develop, form branch rhizomes (unless borne at or above the ground line). Figure 7 shows a short section of permanent lateral root bearing a branched rhizome, the upper portions of which are leafy stems above the ground line (*J*).

The rate of development of the root system under known soil and climatic conditions was observed (in 1937) by means of eleven plants excavated weekly between April 16 and June 24 and a twelfth and a thirteenth plant excavated July 9 and in late October, respectively. A fourteenth plant was excavated in late July, 1939. Figures 1, 2, 3, 4, 8, and 9 show the general vertical penetration and radial spread of representative plants. Data of the fourteen excavations are given in table 2. These plants emerged on the same date, and the first eleven excavations were separated by the same interval of time; yet differentials in the rate of growth caused unequal degrees of development in the series; for example, the plant taken on the eleventh week appears to have reached a stage of development beyond what would be indicated by the plant taken 10 weeks after emergence.

Discussion

The rate of growth of plants of field bindweed from seed under known soil and climatic conditions, with little or no competition, is not to be construed as representing their growth rate under all conditions. It illustrates only the growth potential under this favorable noncompetitive situation.

No soil layer was found which would seriously impede vertical penetration of the roots. However, the roots were smaller in diameter in the 34–39-inch portion of the 21–39-inch interval than they were in the 15 inches above or below this portion. In the study made the 120th week after emergence, practically all the vertical roots observed penetrated this 34–39-inch layer, and the only modification noted was the smaller diameter in the layer. Apparently the general nature and rate of growth found in these studies is representative.

The plants obtained the fourth, fifth, sixth, and seventh weeks after emergence were not secured in their entirety, particularly the full vertical penetration of their roots, owing to the methods used in excavation. Consequently, the experiment for the first 7 weeks was repeated the spring of 1942, which was a better growing season because of more favorable moisture relations. The findings were similar, except in regard to maximum vertical penetration and radial spread. The plant 4 weeks after emergence penetrated vertically $11\frac{3}{4}$ inches with a $4\frac{1}{4}$-inch radial spread; the fifth-week plant reached a depth of $14\frac{1}{2}$ inches with a radial spread of 8 inches; the sixth-week plant extended $17\frac{1}{2}$ inches vertically and 10 inches radially; while the measurements for the plant 7 weeks after emergence were $19\frac{1}{2}$ and $12\frac{1}{2}$ inches, respectively.

KENNEDY and CRAFTS (6) state that the lateral roots which develop extensively to become permanent lateral roots are those more favorably located. This investigation offers no better explanation.

Summary

1. Field bindweed plants were grown from seed in a typical upland loam soil at Manhattan, Kansas, under known temperature and precipitation conditions, and not subject to competition. The plants were studied at various ages, from the seedling stage through 120 weeks of growth, to determine their nature and rate of development.

2. The root system consisted of the original root (primary vertical root) and one to many permanent lateral roots, the vertical extensions of which are termed secondary vertical roots. Unless injury or too severe competition prevents, there are many additional permanent lateral and secondary vertical roots of the second and higher orders.

3. The plant spreads horizontally by means of series of permanent lateral roots. The primary permanent lateral roots arise on the primary vertical root. Succeeding orders of permanent lateral roots arise at the bend where a permanent lateral root of the preceding order turns down to become a vertical root.

4. The plants spread radially, by means of a series of lateral roots, 10⅓ feet in one growing season and 17 feet by the middle of the third growing season, at which time many vertical roots had extended 14–16 feet deep, and one was traced to a depth of 23 feet.

5. The source of shoot development other than that arising from the plumule was determined as root-borne buds which form leafy shoots directly (if at or above the ground line) or rhizomes (if below ground), which in turn give rise to leafy shoots. These arose in greatest abundance at the bend separating the lateral root of any order from its vertical phase. The shoot development of old plants is wholly from root-borne buds.

The writer is indebted to members of the departments of botany of the University of Chicago and of the Kansas Agricultural Experiment Station for helpful suggestions during the course of this study.

DEPARTMENT OF BOTANY
KANSAS AGRICULTURAL EXPERIMENT STATION
MANHATTAN, KANSAS

LITERATURE CITED

1. BAKKE, A. L., Experiments on the control of European bindweed (*Convolvulus arvensis* L.). Iowa Agr. Exp. Sta. Res. Bull. 259. 1939.
2. GUNTHER, R. T., The Greek Herbal of Dioscorides. University Press, Oxford. 1934.
3. HEGI, GUSTAV, Illustrierte Flora von Mittel-Europa. 5:2086–2089. München. Undated.
4. HITCHCOCK, A. S., and CLOTHIER, G. L., Fifth report on Kansas weeds: Vegetative propagation of perennial weeds. Kansas Agr. Exp. Sta. Bull. 76. 1898.
5. IRMISCH, THILO., Ueber die Keimung und die Erneuerungsweise von *Convolvulus sepium* und *C. arvensis*, so wie über hypokotylische Adventivknospen bei krautartigen phanerogamen Pflanzen. Bot. Zeitung 15:433–443. 1857.
6. KENNEDY, P. B., and CRAFTS, A. S., The anatomy of *Convolvulus arvensis*, wild morning-glory or field bindweed. Hilgardia 5:591–622. 1931.
7. KIESSELBACH, T. A., PETERSEN, N. F., and BURR, W. W., Bindweeds and their control. Nebraska Agr. Exp. Sta. Bull. 287. 1934.
8. KILTZ, B. F., Perennial weeds which spread vegetatively. Jour. Amer. Soc. Agron. 22:216–234. 1930.
9. LUND, SAMSØE, and ROSTRUP, E., Marktidselem, *Cirsium arvense* en monografi. Kongl. Danske Vidensk. Selsk. Skr., 6R., Naturv.-Math. Afd. 10:1–152. 1901. (French résumé, pp. 153–165.)
10. ROGERS, C. F., DURRELL, L. W., and DANIELS, L. B., Three important perennial weeds of Colorado. Colorado Exp. Sta. Bull. 313. 1926.
11. SACHS, JULIUS VON, History of botany (1530–1860). Clarendon Press, Oxford. 1906.
12. WEAVER, J. E., Root development of field crops. McGraw-Hill, New York. 1926.

DEVELOPMENTAL ANATOMY OF THE FRUIT OF THE DEGLET NOOR DATE[1]

E. M. LONG[2]

(WITH NINETEEN FIGURES)

Introduction

Quality, as well as size, of mature fruit of the Deglet Noor date is influenced by cultural operations, during the period of fruit development. NIXON and CRAWFORD (6, 7) showed that heavy, as compared with medium, thinning of the fruit clusters or bunches increased susceptibility of fruit to checking during midsummer but decreased fruit shrivel during ripening. (Checks are minute cracks which form in the cuticle and extend through the epidermis and first two to four tiers of the cells of the hypodermal layer. Shrivel appears during ripening, the mesocarp apparently decreasing in volume excessively, so that a wrinkled outer surface develops, as illustrated in figure 8.) ALDRICH *et al.* (1) found that soil-moisture deficiency in June resulted in a greater reduction in rate of fresh- than of dry-weight increase, a reduction in susceptibility of fruit to checking in July and early August, and a tendency for increased shrivel of fruit during ripening. These and other results are more readily interpreted on the basis of chemical and anatomical changes during development.

HAAS and BLISS (4) and RYGG (9) investigated chemical changes during fruit development, but information on the developmental anatomy of the fruit has been incomplete. WINTON and WINTON (13) briefly discussed the structure of the mature fruit, and LLOYD (5) supplied some data on anatomical changes during development, although he was primarily interested in nutrition of the various parts of the fruit. HAAS and BLISS (4) demonstrated that most of the growth in length occurred in the basal region of the fruit, and TURRELL *et al.* (12) studied maturation of the tissue layers of the exocarp in relation to fungus spoilage.

Material and methods

The carpellate inflorescence of the date is a branched spadix composed of 25 to 100 or more spikes, 6–36 inches in length, and may have 8,000–10,000 flowers (8). Material was obtained from inflorescences pollinated March 18, 1941, at weekly intervals from four vigorous young palms at the U.S. Date Garden at Indio, California. At the time of pollination many spikes were entirely removed and several inches taken off the ends of those remaining.

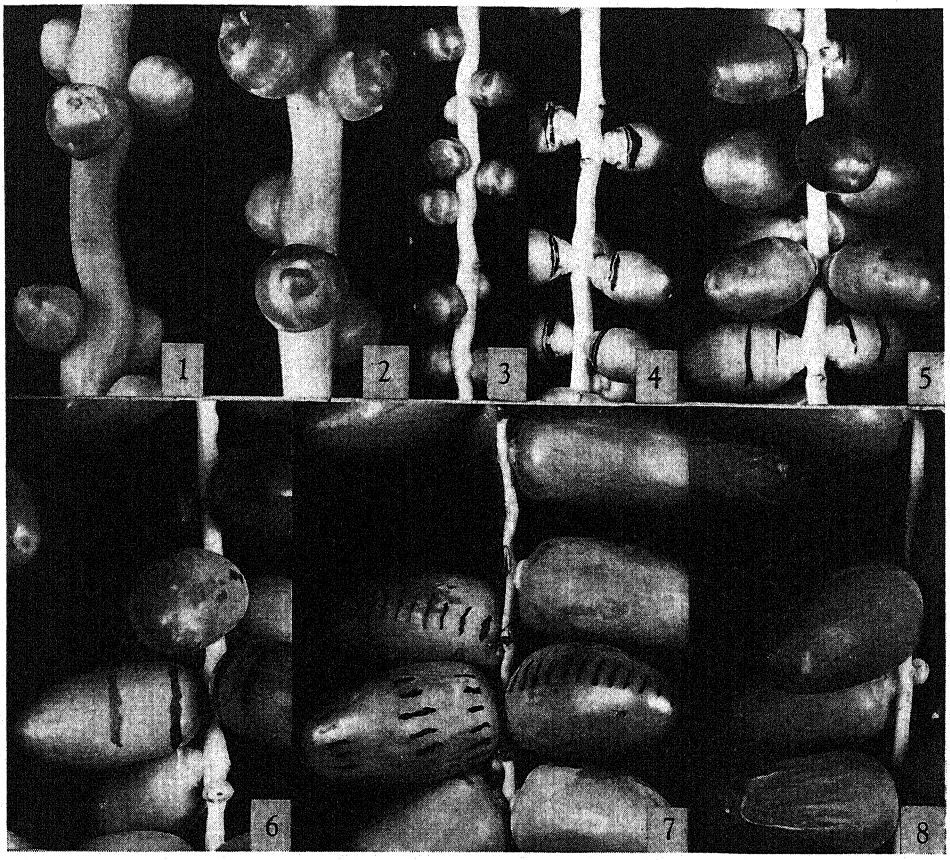
Available soil moisture was maintained by irrigation during the period of active growth. A representative strand was photographed each week for a record. The material for anatomical analysis was fixed in Navashin's solution, dehydrated in an ethyl-tertiary butyl alcohol series, and infiltrated and imbedded with a paraffin-beeswax-rubber mixture. Immediately after placing in the fixative, air was evacuated from the tissues with a vacuum pump. The material was sec-

[1] This study was financed by the United States Date Garden, Indio, California, and was prosecuted under the joint direction of Dr. W. W. ALDRICH, now of the U.S. Horticultural Field Laboratory, Riverside, California, and Dr. H. E. HAYWARD of the U.S. Regional Salinity Laboratory, Riverside, California.

[2] Agent.

tioned at 6–10 μ and stained by ESAU'S (3) modification of the iron-alum haematoxylin schedule.[3]

meristem two or possibly more years previous to spathe emergence. The apical bud is found several feet below the



FIGS. 1–8.—Carpellate flowers and fruits from soon after pollination to fruit ripening: 1, March 27 (early in period I); 2, April 17 (late I); 3, May 8 (early II); 4, May 29 (early III); 5, June 12 (late III); 6, July 17 (early V); 7, August 28 (early VI); 8, October 12 (early VII). Period numbers as shown in fig. 21. All marks on fruits shown in figs. 4 and 5 and mark near equator of fruit in fig. 6 made with ink May 28; mark near base of fruit in fig. 6 made June 19. All marks on fruits in fig. 7 made $\frac{1}{8}$ inch apart on June 26.

Results

FIELD OBSERVATIONS

The inflorescences are borne in large, tough, leathery spathes which emerge from the axils of leaves expanded the previous year, the primordia of which must have originated from the apical level of the expanding leaves, closely and completely surrounded by their sheathing bases, which render the bud almost inaccessible. The carpellate flower is trimerous and ivory white at emergence. The three carpels are not adnate but closely appressed and almost wholly inclosed by a coriaceous corolla composed of three closely imbricated petals (fig. 1).

[3] Most of the slides were prepared by Mr. ARTHUR SPURR.

The calyx is also coriaceous and composed of three thick sepals, of which only the tips are diverged. After emergence, the color of the flower changes through cream and yellow to green.

In 1941, growth was very slow during the first 4 weeks after pollination. By the end of that period one carpel usually became ascendant over the other two, which aborted (fig. 2). During the fifth week after pollination the dominant carpel began to enlarge (fig. 3), and as it emerged from the surrounding perianth, the calyx was split at one or more points.

To demonstrate the relative growth rates in different regions of the carpel, fruits were marked on May 28, the end of the tenth week after pollination, with India ink (4). A heavy black line was made at the base of the carpel, overlapping the distal margin of the corolla. Subsequent growth at the base separated the portion of the mark on the carpel from that on the corolla. The fruits in figures 4 and 5 were photographed 1 and 14 days, respectively, after marking and demonstrate the rapid enlargement during the eleventh and twelfth weeks after pollination. Although growth in length occurred both above and below the marks, by far the greater proportionate longitudinal extension was in the basal region.

Several fruits that had been marked on May 28 were marked again (ink overlapping both carpel and corolla) on June 19. Between June 19 and July 17 (fourteenth through seventeenth week) both median and basal portions of the fruit grew in length, the basal more than the median portion (cf. figs. 5, 6). The distance between the mark of June 19 and the tops of the corolla increased at a diminishing rate until about August 28.

By August 28 the fruits (fig. 7) had attained maximum size and the pink color characteristic of the "khalal" or preripe stage of the Deglet Noor variety. The marks on the fruits shown in figure 7 had been made $\frac{1}{8}$ inch apart on June 26. On August 28 there was no measurable increase in circumference in the apical portion of the fruit after June 26. Growth in circumference was about equal in both median and basal portions. Growth in length during the June 26–August 28 period occurred only in the basal two-thirds to three-fourths, with progressively less growth at increasing distances from the perianth.

During September the fruit decreased in size as a result of dehydration incident to ripening (1, 4, 9). As the pink color changed to brown, softening began at the tip. Fruits on the same strand began to soften at different times during a 2-month period beginning the last week in September. The fruit in the lower part of figure 8 was ripening. It shows some shrivel, which is commercially undesirable but typical for the district in which this fruit was grown.

Detailed observations

Six rudimentary stamens regularly occurred in carpellate flowers, although no anthers were observed. At time of pollination, comparatively little cellular differentiation was evident in the carpel (figs. 9, 10). Differentiation, beginning at the apical end, progressed slowly for 1 month thereafter, then more rapidly. By the seventh week all tissue layers were well differentiated at the equatorial region (fig. 11), and cell division in the mesocarp at that level had been completed, further growth of that tissue being accounted for wholly by cell enlargement.

To encompass the increase in volume of mesocarp from the seventh week to attainment of maximum size, the exo-

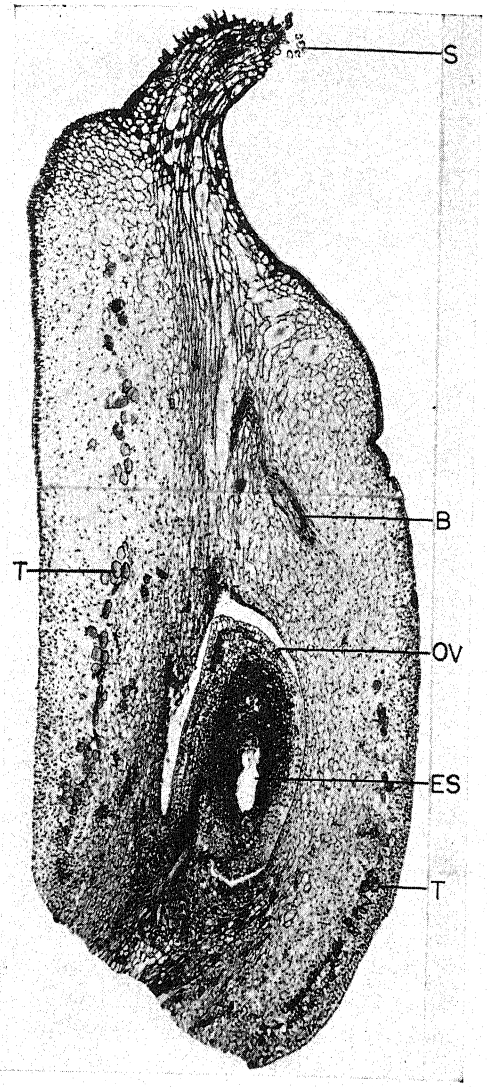


FIG. 9.—Nearly median longisection of carpel at pollination, March 18 (period I): *s*, stigma; *b*, abaxial bundle; *ov*, ovule; *es*, embryo sac; *t*, tannin cells.

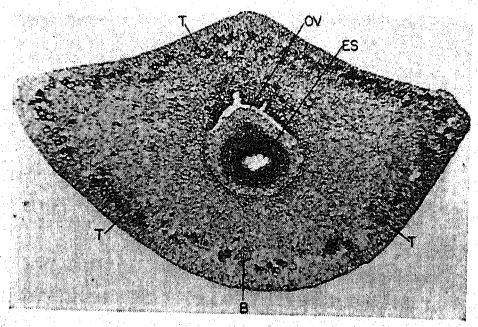


FIG. 10.—Transection of carpel at level of embryo sac.

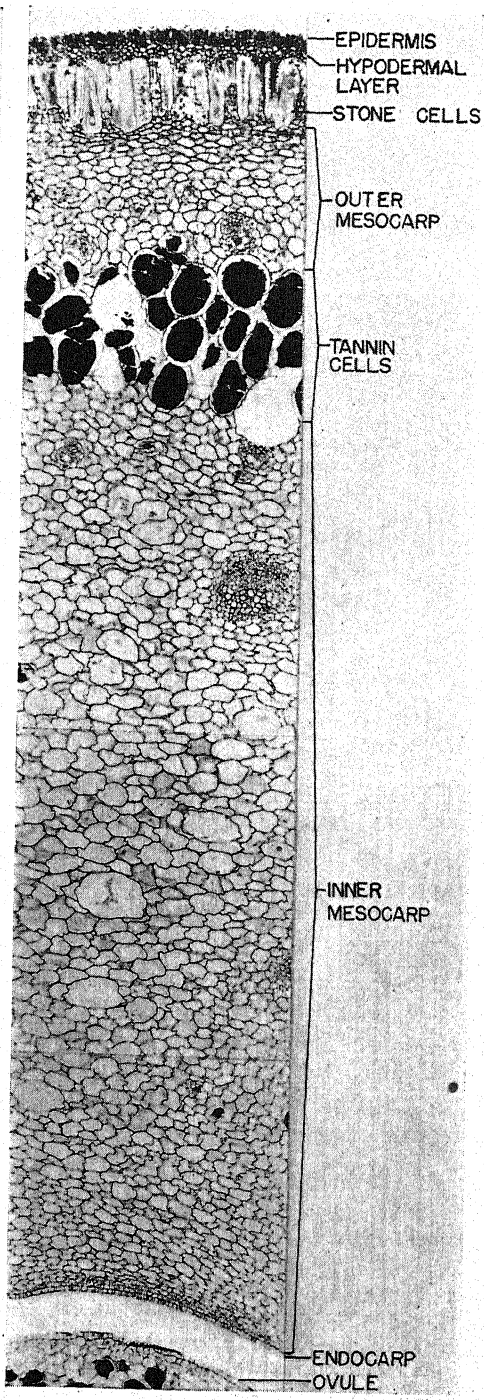
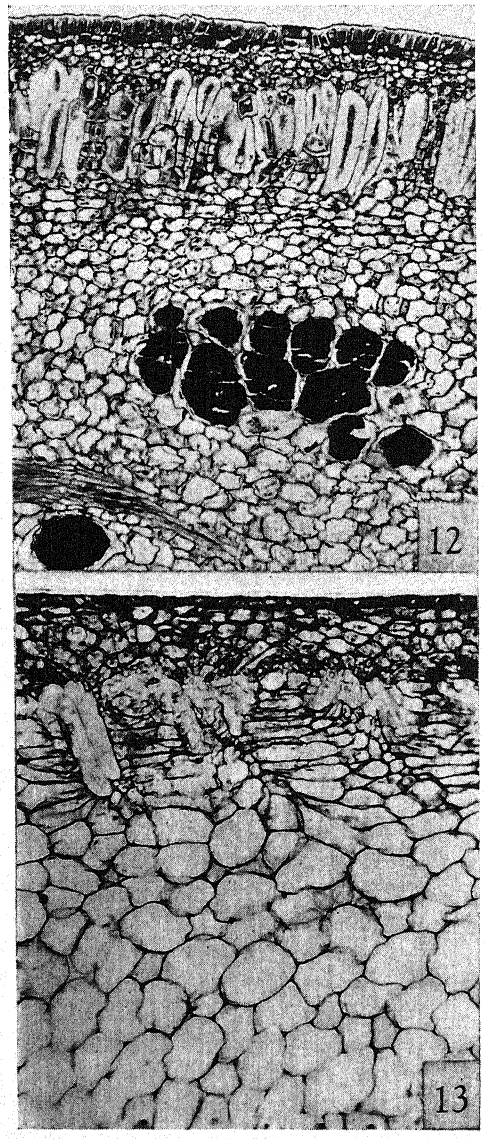


FIG. 11.—Sector of transection through pericarp from equatorial region of fruit collected May 8 (7 weeks after pollination) shortly after completion of cell division in inner mesocarp at this level.

carp must increase in circumference by at least 200 per cent. Longitudinal growth of the exocarp in this zone could not be accurately measured but must have been considerably greater than growth in circumference, for cells of the mesocarp grew more in the longitudinal than in either transverse dimension. Also, the minute cracks or checks which may occur in these fruits during rainy or humid weather from mid-June to mid-August are always transverse, seeming to indicate that in this variety tension on the cuticle where the cracks originate (2) is greater in the longitudinal direction.

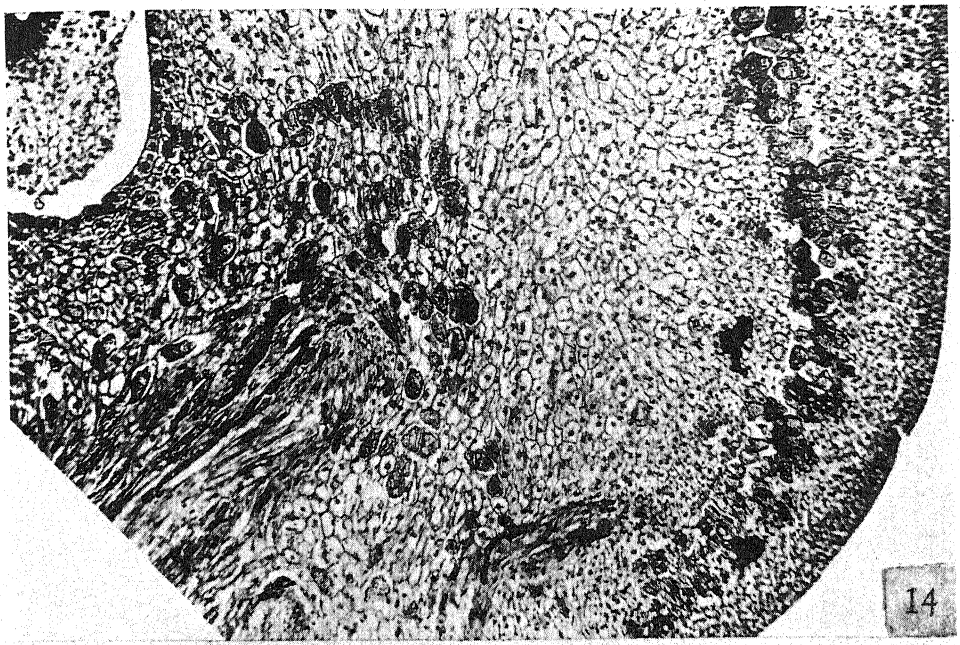


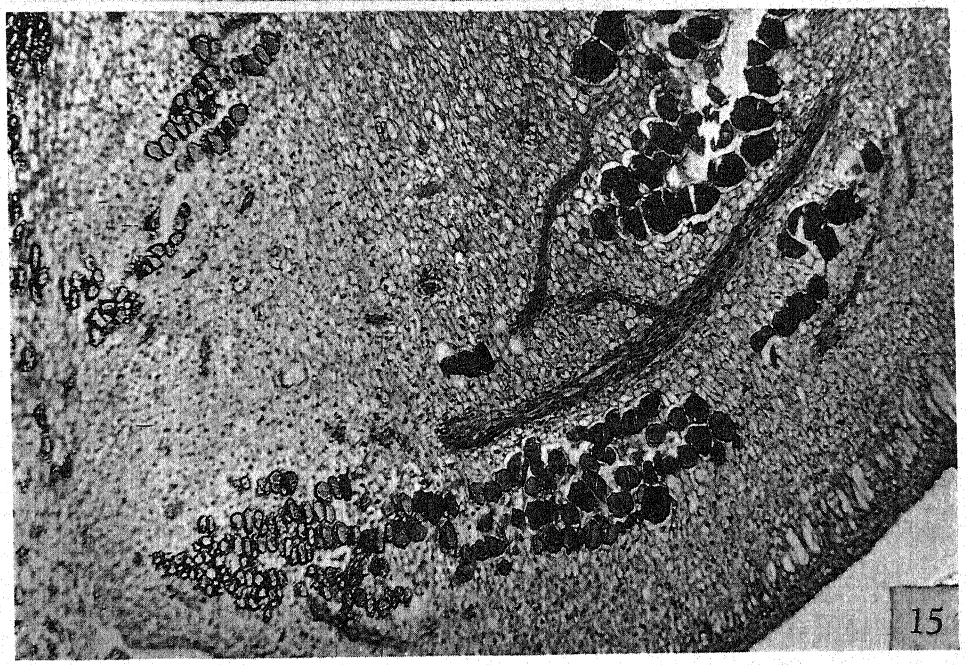
FIGS. 12, 13.—Fig. 12, exocarp and outer mesocarp in radial longisection from equatorial zone of fruit collected May 15. Fig. 13, exocarp and portion of outer mesocarp in radial longisection from apical third of fruit collected September 4, at approximately maximum size, showing extension in stone cell layer accomplished mainly by "stretching" of intervening parenchymatous cells.

Comparison of figures 12 and 13 shows how the exocarp grew in regions of the fruit where tissues were well differentiated. In the epidermal and hypodermal layers both cell division and cell enlargement took place. After stone cells began to mature in a given region, no new stone cells were formed, but the intervening parenchymatous cells elongated greatly, isolating clusters of stone cells. Orientation of many of the stone cells also appeared to change during extension in the exocarp.

Through the first month after pollination little mitotic activity was apparent (fig. 14), and the relatively few cell divisions that did occur were distributed throughout the carpel. This contrasts sharply with the aspect of the basal region 2 weeks later (fig. 15), shortly after organization at the base of the carpel of an actively meristematic zone. Continued activity in this meristem gave rise to cells which constituted more than three-fourths of the mature pericarp.

In some fruits the basipetal meristem ceased activity by the middle of June, while in others it continued active for as much as another month. This was the first indication of a deviation in development of a fruit destined to mature early as contrasted with a later-ripening one. No reason for this difference was appar-

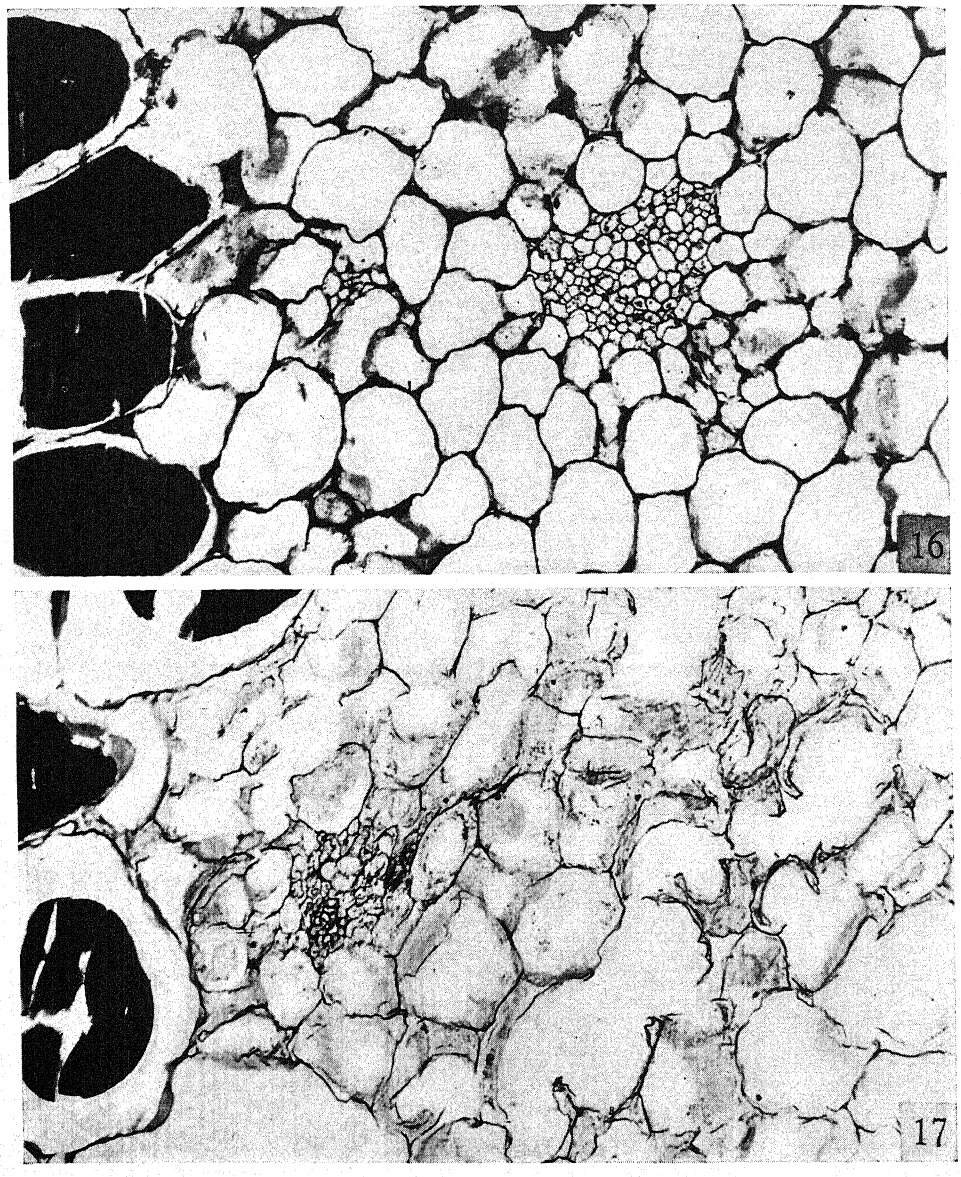




FIGS. 14, 15.—Fig. 14, radial longisection through basal region of fruit collected April 17, showing no evidence of recent, very active cell division, although scattered cell divisions occur throughout carpel at this stage (late period I). Fig. 15, radial longisection through basal region of fruit collected May 1, soon after beginning of activity of basipetal meristem, showing evidence of rapid successive divisions of cells of basal zone.

ent. About the middle of July, cells of the basipetal meristem of even the latest maturing fruits ceased to divide, enlarged, and finally matured.

Besides the color change already mentioned, the most apparent outward evidence of ripening is softening, accompanied by dehydration, so that the fruit becomes somewhat flaccid. The change in texture is due in part to the fact that



FIGS. 16, 17.—Partial dissolution of cell walls which contributes to softening of fruit during ripening. Fig. 16, mesocarp in transection from equatorial zone of fruit collected August 21, nearly full size but immature; cell walls relatively thick and strong (late period V). Fig. 17, comparable tissues of ripe fruit collected October 10; walls thin and often broken (period VII).

the walls of the mesocarp cells become thinner and weaker, apparently being hydrolyzed, until some of them undergo almost complete disintegration (figs. 16, 17). Cell membranes apparently lose the property of differential permeability before microscopic evidence of wall dissolution is apparent (2). Softening begins at the apex of the fruit, progressing toward the base, and also from the periphery toward the endocarp.

CELL COUNTS AND MEASUREMENTS.—To determine the time at which periclinal divisions ceased in the terminal segment of the fruit, cell size was plotted against breadth of mesocarp (fig. 18*B*). This method was suggested by SINNOTT (10), who states the theoretical relationship between cell size and organ size (expressed graphically in fig. 18*A*) thus: "the cessation of cell division is associated with the attainment of a definite cell size. . . . . Increase in cell size is not a continuous process, but is interrupted at each cell division, for cell volume is then reduced by half and cell diameter by slightly more than 20 per cent. . . . . Each daughter cell now increases . . . . to a somewhat greater diameter than that of its mother cell and then divides again. Thus the changes in size in a given cell lineage are not to be represented by a continuous line but by a serrate one. . . . . After the last division, cell enlargement proceeds continuously, uninterrupted by further breaks, at approximately the same rate as fruit enlargement."

In obtaining the data from which curve *B* of figure 18 was plotted, the distance from endocarp to the tannin cells (fig. 11) opposite the chalazal end of the ovule was measured with an eyepiece micrometer. Then the number of cells intersected by the edge of the micrometer scale in the distance measured was counted. Thus many cells were measured at tapered ends, while SINNOTT (10) arbitrarily selected for measurement cells judged to be "a common maximum size . . . . characteristic of the whole area." Owing to the considerable variation in size of cells selected at random, it was necessary to measure many in order to obtain reproducible values for average cell diameters. To compensate for distortion due to compression of the material during sectioning, all counts and measurements were made once in the direction parallel to the knife edge at

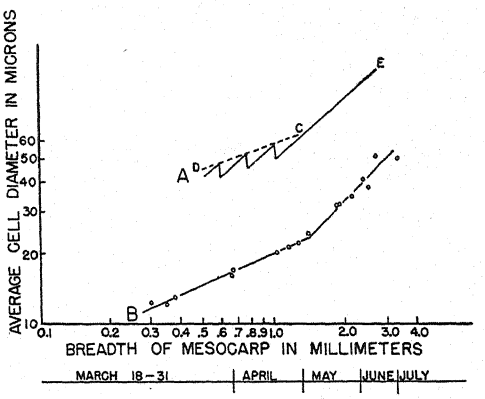


FIG. 18.—Relation of cell diameter to breadth of mesocarp plotted logarithmically. *A*, theoretical curve, after SINNOTT (10). *B*, actual curve for apical segment.

sectioning and again in the direction at right angles to the knife. The values represented by one point in figure 18 are averages of two such counts and measurements for each of three to six fruits, and they include at least 150 cells.

The slope of line *CE* in figure 18*A* is that produced when all the growth of an organ is the result of enlargement of a constant number of constituent cells. In curve *B*, previous to early May the slope is considerably less than that of *CE* (much closer to that of *DC*), indicating that increase in breadth of mesocarp at that time resulted from increase in both number and size of cells. The break in curve *B* in early May, and the subse-

quent slope paralleling *CE*, indicate that after that time all growth in breadth of the mesocarp in the vicinity of the distal end of the seed was due entirely to cell enlargement.

The basipetal growth of the fruit results in so few permanent longitudinally distributed reference points as to make it impossible to locate precisely analogous tissue for study at progressive intervals throughout the season. Since in fruits collected at different times, one could not identify for measurement tissues laid down during any specific period, SINNOTT's method could not be used to determine the date at which transverse division ceased in any given segment. However, the assumption that "cessation of divisions is associated with the attainment of definite cell size" (10), coupled with adequate cytological observations, permitted rather accurate determination of the role of such divisions in longitudinal growth. To determine growth of cells at any given level, the eyepiece-micrometer scale was superimposed on the middle of the inner layer of mesocarp of a fruit in radial longisection and counts made of the number of cells intersected by the edge of the scale in a measured distance. For each determination a minimum of fifty cells from three to six fruits from one sample were measured. Largely upon the basis of data so obtained (fig. 19), growth and development of the date fruit has been divided into seven periods. TUKEY and YOUNG (11) by a somewhat similar method have divided the development of the fruit of the sour cherry into three stages.

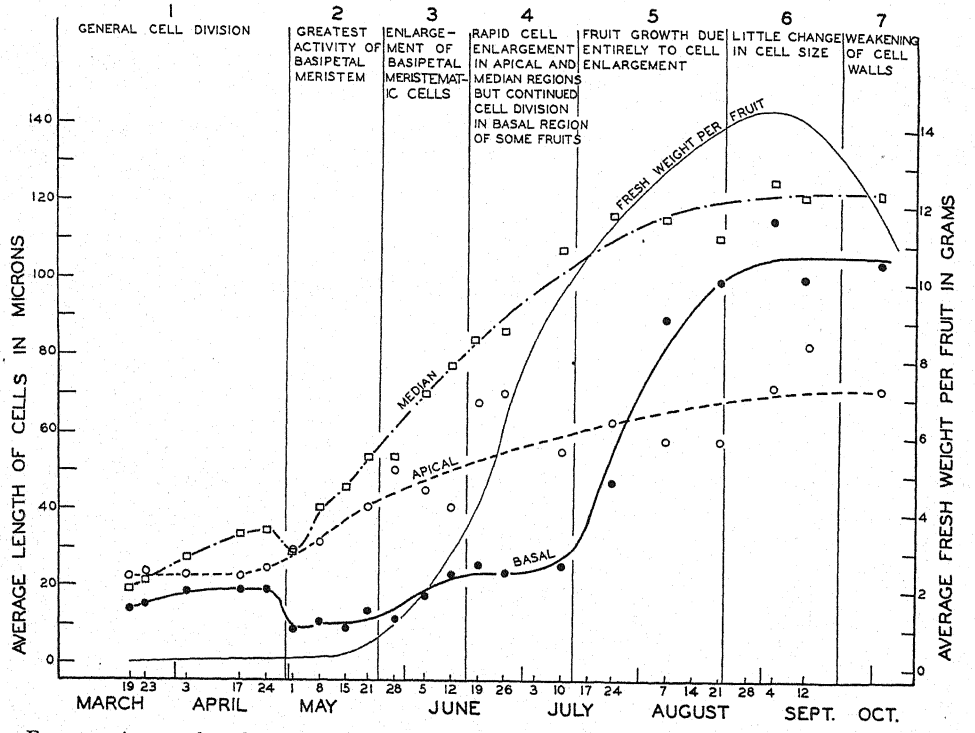


FIG. 19.—Average lengths of cells of basal, median, and apical regions of mesocarp, and fresh weight of average fruits; determinations from pollination to maturation. Data for fresh weight curve provided by W. W. ALDRICH and C. L. CRAWFORD of the U.S. Date Garden.

The curves representing cell length in the basal, median, and apical regions of the mesocarp during period I were more or less similar to *DC* in curve *A* of figure 18, indicating that some transverse cell division was occurring throughout that layer of the pericarp. The second period was initiated by rapid successive transverse divisions of the cells in the basal end of the fruit, resulting in much shorter cells in that region, which then constituted a basipetal meristematic zone (fig. 15). Since the area of active cell division extended at first almost to the equator of the young fruit, the lines representing lengths of both median and basal cells dipped, while apical cell length increased uninterruptedly. Cells in the median zone were rapidly separated from the basipetal meristem by growth of intervening cells; and, after a short phase of mitotic activity early in the second period, they ceased division. During the latter part of period II the endosperm, which had previously been free-nuclear, became cellular.

During period III the cells of the basipetal meristem increased in length, while continuing active division. The end of the period is marked by cessation of division in some fruits, which is associated with and identified by attainment of a cell length of about 30 μ in the basal zone. Thus period IV is initiated by the disappearance of the basipetal meristematic zone in the fruits destined to mature earliest and ends with the cessation of cell division in the basal zones of the latest maturing fruits. Period IV is characterized by the most rapid fresh-weight increase as well as by the beginning maturation of previously meristematic cells. During period V there is no cell division and all increase in the volume of the mesocarp is due to cell enlargement, chiefly of the cells of the basal region.

The stages of fruit development corresponding to periods IV and V have been found by HAAS and BLISS (4) and by ALDRICH *et al.* (2) to be those during which the fruits are susceptible to checking. Period V corresponds to RYGG's (9) stage 2, which he found to be characterized biochemically by a greatly reduced rate of accumulation of reducing sugars and a high degree of hydration.

Period VI, which corresponds in a general way to the "khalal" or preripe stage, is one of little apparent anatomical change. RYGG found that rapid sucrose accumulation and slow dehydration occur at this stage. The fruit is no longer susceptible to checking (2, 4), but —if it comes into contact with free water —is subject to tearing, that is, the fruit bursts open as a result of excessive turgor, separation occurs at the tannin cell layer, and segments of the exocarp and outer mesocarp break away from the remainder of the fruit, curling outward at the edges of the rupture.

Period VII is characterized by weakening of cell walls, apparently as a result of hydrolysis (fig. 17), a color change from pink to brown, further increases in sucrose (9), and rapid dehydration. Since dehydration decreases the size of the fruit during maturation, at least some of the cells must also have decreased in size. This is not apparent from figure 19, because the material was dehydrated in the histological procedure. Since histological dehydration shrank tissues and constituent cells before they were measured microscopically, actual maximum size of cells was never measured. The decrease in fresh weight, due to water loss during ripening and previous to collection, did not significantly influence values obtained in measuring cell lengths, because the removal of water and consequent volume loss would have occurred during the histological procedure if not previous to it.

Summary

1. The development of the fruit of the Deglet Noor date from pollination to maturation in 1941 was divided into seven periods. The calendar dates for each period refer to the fruits examined in 1941 and may be expected to vary somewhat with the district where grown and in different growing seasons.

2. Period I, from March 18 (pollination) to April 30, is an interval of little enlargement, during which some cell division occurs throughout the mesocarp. Period II, May 1 to 23, is initiated by organization of a meristematic zone at the base of the fruits. Cell division ceased in the apical half of the mesocarp by May 8. At the close of this period the endosperm, previously free-nuclear, became cellular. Period III, May 24 to June 15, is initiated by a sharp increase in rate of enlargement of the entire fruit and all cells of the mesocarp, including those of the basipetal meristem, which, however, continue to divide. At the close of this period the fruits become susceptible to checking (minute cracks involving the cuticle, epidermis, and outer cells of the hypodermal layer). Period IV, June 16 to July 13, begins with inactivation of the basipetal meristem of fruits destined to mature earliest and ends when a meristematic zone is no longer present in any of the fruits. This period is also characterized by the highest rate of fresh-weight increase of the season. Period V, July 14 to August 22, starts with a marked increase in rate of elongation of the previously meristematic cells at the base of the fruits and ends as those cells approach maximum length. At the end of this period the fruit is no longer susceptible to checking, and the color changes from green to pink. Period VI, August 23 to September 21, is distinguished by little apparent anatomical change. Marked increase in percentage of dry matter occurs. Period VII, September 22 to November 3, is characterized by softening as a result of continued dehydration of the fruit and partial dissolution of cell walls. The color changes from pink to brown.

U.S. DATE GARDEN
INDIO, CALIFORNIA

LITERATURE CITED

1. ALDRICH, W. W., CRAWFORD, C. L., and MOORE, D. C., Some effects of soil moisture deficiency at different times upon the size and quality of fruit of the Deglet Noor date. U.S.D.A. Tech. Bull. (in press) 1943.
2. ALDRICH, W. W., FURR, J. R., CRAWFORD, C. L., and MOORE, D. C., Checking of Deglet Noor date fruits in relation to water deficits in the palm. Plant Physiol. (in press) 1943.
3. ESAU, KATHERINE, Phloem anatomy of tobacco affected with curly top and mosaic. Hilgardia **13**:437–470. 1941.
4. HAAS, A. R. C., and BLISS, D. E., Growth and composition of Deglet Noor dates in relation to water injury. Hilgardia **9**:295–344. 1935.
5. LLOYD, F. E., Development and nutrition of the embryo, seed and carpel in the date, *Phoenix dactylifera* L. Rept. Missouri Bot. Gard. **21**:103–164. 1910.
6. NIXON, R. W., and CRAWFORD, C. L., Fruit thinning experiments with Deglet Noor dates. Proc. Amer. Soc. Hort. Sci. **34**:107–115. 1937.
7. ———, and ———, Quality of Deglet Noor date fruits as influenced by bunch thinning. Proc. Amer. Soc. Hort. Sci. **40**:103–110. 1942.
8. ROBBINS, W. W., The botany of crop plants. 3d ed. Blakiston's. 1931.
9. RYGG, G. L., Compositional changes in the date fruit during growth and ripening. U.S.D.A. Tech. Bull. (in press).
10. SINNOTT, E. W., A developmental analysis of the relation between cell size and fruit size in cucurbits. Amer. Jour. Bot. **26**:179–189. 1939.
11. TUKEY, H. B., and YOUNG, J. O., Histological study of the developing fruit of the sour cherry. BOT. GAZ. **100**:723–749. 1939.
12. TURRELL, F. M., SINCLAIR, W. B., and BLISS, D. E., Structural and chemical factors in relation to fungus spoilage of dates. Rept. Date Growers' Inst. **17**:5–11. 1940.
13. WINTON, A. L., and WINTON, KATE B., Structure and composition of foods. Vol. II. Wiley and Sons. 1935.

DEVELOPMENT OF SORUS AND PROTHALLIUM OF ACROPHORUS STIPELLATUS

BETTY F. THOMSON

(WITH TWENTY-NINE FIGURES)

Introduction

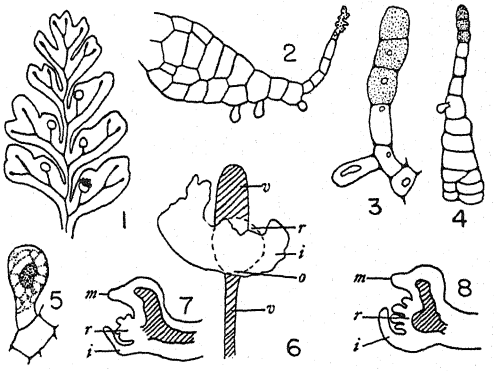
The fern now known as *Acrophorus stipellatus* (Wall.) Moore has been placed in various genera of the Polypodiaceae, including *Davallia*, *Aspidium*, and *Cystopteris*. BOWER (2) discusses it among the Dryopteroids but considers it to be a *genus incertae sedis*. This uncertainty as to its relationships is due chiefly to lack of exact information about the development of the sorus.

The material used in this study was collected in July, 1937, by Professor ALMA G. STOKEY in Java. The plants were growing in rich damp soil at an altitude of 2400 meters. Some specimens were dried and pressed, others were killed and fixed in a formalin-acetic acid-alcohol solution. At the same time prothallial cultures on sterile peat were started from spores. Material for detailed study was later imbedded in paraffin, sectioned at 12 μ, and stained with safranin and fast green or with iron-alum haematoxylin. Living prothallia were also examined whole.

Observations

ADULT PLANT

The plants have an erect stem and a basket-like habit. The leaves are about 1 meter long, quadripinnate, with open, dichotomous venation (fig. 1). Each veinlet ends as an enlarged mass of tracheids which forms an elliptical hump on the under side of the pinnule. The shoot is luxuriantly clothed with hairs and scales of varying degrees of complexity, many of which are glandular. Simple glandular papillate hairs may be found on the leaf surfaces and rachises, and the more complex hairs and scales may have glandular tips or appendages (figs. 2–5).



FIGS. 1–8.—Fig. 1, portion of pinnule showing form, venation, and location of sori. Figs. 2–5, dermal appendages; glandular cells shaded; figs. 3 and 5 more highly magnified. Fig. 6, sorus with sporangia removed showing vein extending beyond receptacle: *v*, vein; *r*, receptacle; *i*, indusium; *o*, point of origin of indusium. Figs. 7, 8, adjacent sections of young sorus, cut parallel to vein, showing receptacle supplied by short branch from vein; vascular tissue shaded; hairs and scales omitted: *m*, recurved leaf margin; *r*, receptacle; *i*, indusium.

Fertile and sterile leaves are alike in their general aspect. Mature sori, one to eight on a pinnule, are situated on the veinlets just short of their swollen ends. The vein may obviously extend past the sorus, or the sorus may appear to cover the entire vein ending. When the sporangia were removed, however, in every specimen examined the vein was found to continue past the receptacle, even when the sporangia extended out in a pompon-

like cluster in such a manner that they concealed the vein ending completely (fig. 6). A branch from the vein passes into the convex, zygomorphic receptacle, where it ends as a slightly enlarged mass of tracheids similar in structure to the enlarged ends of the veinlets in the vegetative parts of the leaf (figs. 7, 8). The basally attached indusium completely covers the young sorus; but as the sporangia enlarge and their stalks elongate, they push the protective scale up and back until it forms an incomplete covering for the sorus.

Development of sorus

The first recognizable indication of a sorus appears when the pinnule is still very small. In cross-section the young leaflet is elliptical, about twice as broad as thick, when the first indications of the vascular strand can be distinguished. At this stage the cells immediately behind the marginal cell on the abaxial side show anticlinal divisions (fig. 10). After further divisions of this nature and growth of the resulting cells, and with the establishment and enlargement of the indusial initial, the young fertile leaflet suggests in section an isosceles triangle, with the apex formed by the marginal initial of the sterile side and the basal corners formed by the large indusial initial and the marginal initial of the fertile side (figs. 11, 12). Because of this triangular shape, the receptacle appears at first glance to be marginal, since the receptacle cells formed by anticlinal divisions push the true margin to an asymmetrical position. But the receptacle is as nearly superficial as possible on a structure so little expanded that it has no flat surface. Figure 9 shows a sterile leaflet at an early stage of development for comparison.

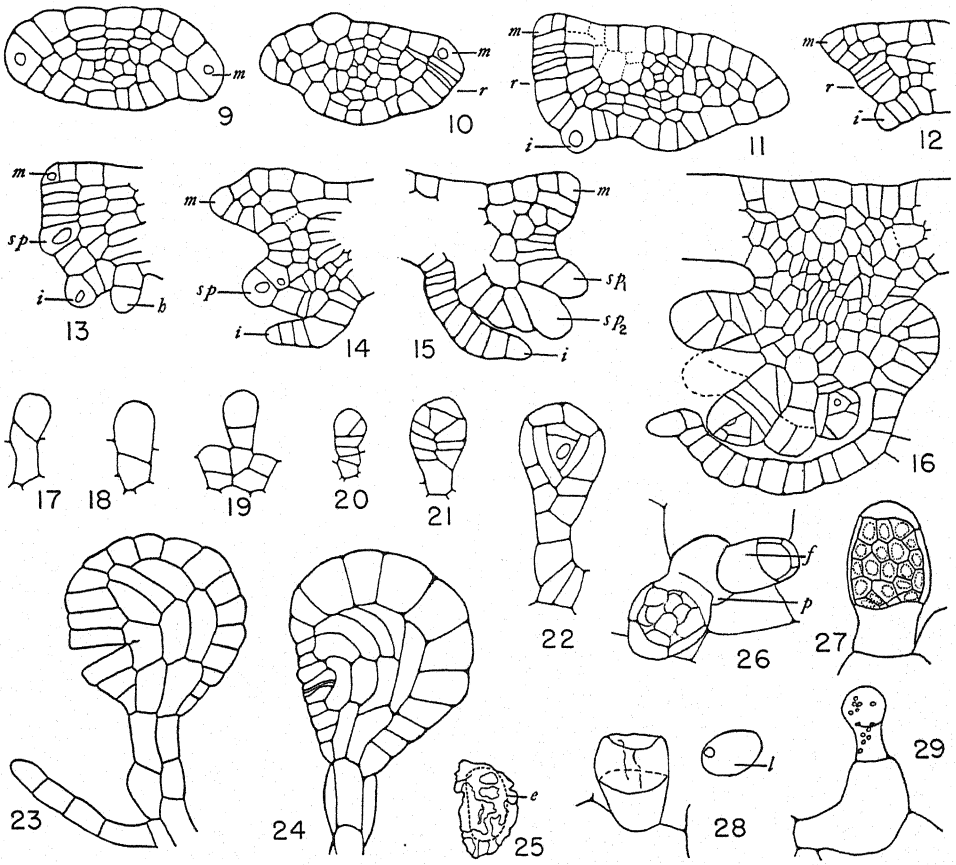
The marginal initial can also be distinguished in later stages (figs. 13–15). No instance was found in which the receptacle could be regarded as truly marginal in origin. In the youngest sorus observed (fig. 10) the latest cell derived from the marginal initial had undergone the anticlinal divisions which lead to development of the receptacle, but in no case was the marginal initial itself involved in the construction of the sorus.

The indusium, developing by means of intercalary divisions, arises superficially just behind the cells which multiply to form the receptacle. It is several cells long as seen in section by the time the first sporangial initial can be distinguished (figs. 13, 14). As the receptacle grows larger and projects farther below the leaf surface, the indusium, with the central part of its base adhering to the receptacle, is carried away from the laminar surface, obscuring the superficial nature of its origin.

The first sporangium arises from the center of the receptacle, which is somewhat convex and tilted toward the leaf margin at this time (figs. 13, 14). The second seems to arise consistently between the first and the indusium (figs. 15, 16). The first four or five sporangia arise in basipetal sequence. The receptacle meanwhile enlarges by means of intercalary divisions and some elongation of the cells at its base and becomes more sharply convex. The sporangia which arise from the later-formed part of the receptacle develop in mixed sequence. They follow one another in such rapid succession that those of a single sorus are all at approximately the same stage of development. The sporangial initial is more or less square at the base as seen in longitudinal section. The first segmenting wall may be transverse but is more often oblique, abutting on a lateral wall of the initial cell, generally—but not al-

ways—below the level of the surface of the receptacle (figs. 17–22). More sporangia are formed on the side of the receptacle toward the indusium than on that toward the leaf margin. Any cell on the surface of the receptacle may become a sporangial initial; in fact, almost every cell over the entire receptacle gives rise to a sporangium, with the result that the sporangia are densely crowded in the mature sorus. Very few paraphyses were found arising from the receptacle; hairs attached to the sporangial stalks are more numerous (fig. 23).

SPORANGIUM AND SPORES.—The mature sporangium is borne on a stalk consisting of three cell rows. The stalk may bear a simple curved hair about four cells long. The capsule is very much like that of *Dryopteris filix-mas* as shown in KNY's *Wandtafeln*. The annulus is vertical and incomplete, being interrupted at



FIGS. 9–29.—Fig. 9, young sterile leaflet, cross section. Figs. 10–16, sections of young fertile leaflets. Fig. 10, earliest stage observed; cells adjacent to marginal initial show anticlinal divisions. Figs. 11–16, later stages: *h*, hair; sp_1, sp_2, first and second sporangial initials. Figs. 17–22, developing sporangia showing variation in initial segmentation. Figs. 23, 24, young sporangia showing unequal faces and hair on sporangial stalk. Fig. 25, spore showing wrinkled perispore: *e*, outline of exine. Fig. 26, two antheridia borne on a pedicel cell (*p*); note cylindrical form of "funnel cell" (*f*). Fig. 27, young antheridium without pedicel cell. Fig. 28, ruptured antheridium with extruded undivided lid cell (*l*). Fig. 29, papillate prothallial hair with chloroplasts, borne on enlarged marginal cell.

the insertion of the stalk (figs. 23, 24). It consists of twelve to fourteen indurated cells and seven to nine thin-walled cells in the stomium region. The stomial cells are elongated transversely and twisted to one side because of the inequality in the numbers of cells on the two faces of the capsule (figs. 23, 24).

The bilateral spores appear black to the eye and golden brown under magnification. The exine is smooth, but the spore is incased in a transparent, loosely-applied membrane or perispore which is folded into an irregular network of ridges, giving the spore a characteristic surface modeling (fig. 25).

During early development of the sorus, the extension of the lamina is delayed, but this lag is subsequently made up, and the blade finally extends far beyond the receptacle. The leaf margin is strongly recurved over the young sorus, which is well developed while the leaf is still tightly rolled. As the leaf unrolls, the blade becomes flattened.

PROTHALLIUM.—Spores germinated slowly. Fifteen weeks after they were sown, when growth was first discernible to the naked eye, the largest prothallia had just established a multicellular meristem. Their uniform growth was considered an indication that the cultures were pure; but the identity of young prothallia was also checked by the modeling of the spore coat, which could usually be found adhering to the basal end of the thallus.

Prothallia are of the usual delicate polypod type. Unicellular papillate hairs are found along the margins of young prothallia and scattered over the surfaces of mature ones. Hairs may arise superficially from any cell, frequently on an enlarged base and protruding from the thallus, especially in the case of marginal hairs (fig. 29).

Antheridia may arise directly from the surface of the thallus (figs. 27, 28), or they may be borne on a subglobose pedicel cell (fig. 26). In some cases two or even three antheridia of different ages arose from a single pedicel cell (fig. 26). The cell which in most polypods is designated as the "funnel cell" is more or less cylindrical rather than funnel-shaped (figs. 26, 27). When the antheridium opens, the undivided lid cell is extruded intact (fig. 28). Archegonia are of the usual polypod type.

Discussion

Acrophorus has been placed by some systematists among ferns of Davallioid or marginal derivation, by others among Cyatheoid or superficial derivatives. As pointed out by BOWER (2), its basket-like habit, its dictyostelic anatomy with a highly segregated leaf trace, and the presence of abundant chaffy scales indicate an affinity with *Dryopteris* and the superficial line.

The association of *Acrophorus* with the Marginales is based on two misconceptions: that the sorus is terminal on the vein and that the receptacle is marginal in origin. It has just been pointed out that the sorus is lateral rather than terminal on the veinlets; sections cut parallel to the vein show that a small extension from the veinlet enters the receptacle, where it ends as a slightly enlarged mass of tracheids (figs. 7, 8). Such an arrangement is unlike that in marginal derivatives, where the sorus, even when superficially placed at maturity, is located on a vein ending. Early stages in sorus development appear at first glance to have originated on the margin of the leaf, but the marginal initial itself is not involved in sorus formation.

Of the Superficiales, *Acrophorus* resembles both the Woodsioid and the

Dryopteroid groups. These two groups show a continuous progression in the development of a zygomorphic indusium, from *Woodsia* (11), with indusial hairs and scales which lag in development on the side toward the leaf margin, to *Peranema* (5), with a scale attached only on the side of the receptacle away from the leaf margin but completely inclosing the entire sorus, and to *Dryopteris*, with a basally attached indusium which has assumed a kidney shape related to the form of the expanded receptacle. *Acrophorus* has advanced with respect to its indusium to the same stage as *Peranema* and *Cystopteris*. The indusium in all three is attached along a broad base, free at the other margins and overarches the sorus completely.

The receptacle in all members of the two groups, including *Acrophorus*, is superficial in origin and situated near the end of a vein, but on a special short branch or extension rather than on the actual vein ending (figs. 6–8). The form of the receptacle is regularly round or hemispherical in the Woodsioideae, except in the case of *Peranema*, where it becomes tilted toward the leaf margin as it develops. In the Dryopteroids the receptacle is tilted from its first appearance and is overarched by the indusium before the first sporangium is initiated. Similar sections of very young fertile pinnules of *Dryopteris* and *Acrophorus* are much alike, except for the decided lag in the extension of the lamina in the latter.

Early stages in soral development of *Cystopteris* are strikingly similar to those of *Acrophorus*. Figures of *C. bulbifera* given by PALSER and BARRICK (10) might have been drawn from preparations of *Acrophorus*. *C. montana* (8) and *C. fragilis* (1) also closely resemble *Acrophorus* in the origin of the receptacle and indusium adjacent to but independent of the retarded leaf margin.

The Woodsioideae show transitions from gradate sporangial development in *Woodsia*, to gradate becoming mixed in *Peranema*, to purely mixed in *Hypoderris* and *Diacalpe*. *Dryopteris* shows gradate development at first but soon passes to the mixed condition. In this *Acrophorus* agrees with both *Peranema* and *Dryopteris* (2), as well as with *Cystopteris* (10). Sporangial segmentation in *Acrophorus* is like that in *Peranema*, in that the first wall dividing the initial cell may be either transverse or oblique (6). The transverse wall is characteristic of higher polypods, the oblique is characteristic of ferns on an evolutionary level with the Cyatheaceae. The mature sporangium is much like that of *Dryopteris filix-mas* in the number and arrangement of cells of the capsule, in the form of the stalk, and in the frequent occurrence of a hair on the stalk. In the presence of a perispore it agrees with most Dryopteroids and Woodsioids, although this structure is lacking in *Cystopteris* (9).

Of prothallial characters, those generally considered to be of phyletic significance are the nature of any hairs present and the condition of the antheridial lid cell. The unicellular hairs on the prothallium of *Acrophorus* closely resemble those described by DAVIE (6) for *Peranema* in their shape and distribution and in the fact that they are often borne on slightly elevated or protruding superficial cells. Such hairs are more like those of *Dryopteris* than those of the Woodsioideae. The antheridial lid cell is undivided, as it is in *W. ilvensis*, *Peranema*, *Cystopteris*, *Dryopteris*, and other higher polypods. The unindented funnel cell and the elevation of one or more antheridia on a globular pedicel cell seem to be somewhat unusual characters. Devel-

opmental stages of prothallia are similar to those figured by DOPP (7) for *Cystopteris fragilis*.

There is a strong resemblance between *Acrophorus* and *Cystopteris;* the chief differences are in size and habit and in the lack of a perispore in *Cystopteris*. It seems probable that these genera, while distinct, are very closely related.

CHRISTENSEN (4) places *Acrophorus* among a group of genera between the Woodsioideae and the Dryopteroideae. The structural details here described support this disposition. The zygomorphy of the sorus of *Acrophorus* from its first appearance and the early establishment of mixed sporangial sequence point to a close affinity with *Dryopteris*, although the unexpanded receptacle and the inconstant manner of segmentation of the sporangial initial indicate a less advanced state for *Acrophorus*.

Summary

1. The sorus of *Acrophorus stipellatus* is not terminal on a vein but is borne on a short branch arising near the vein ending.

2. The sorus originates close to the leaf margin, but the marginal initial cell is never involved in sorus construction. The sorus is zygomorphic from its first appearance. Sporangia arise at first in gradate but soon in mixed sequence.

3. The first segmentation of the sporangial initial may be either transverse or oblique. The sporangial stalk consists of three cell rows and sometimes bears a curved hair. The annulus is vertical and incomplete, with an unequal number of cells in the two faces of the capsule.

4. Prothallia are of the usual polypod type and bear unicellular papillate hairs. The antheridial "funnel cell" has the form of a solid cylinder. The lid cell is extruded intact at maturity.

5. The soral development and structure of *Acrophorus* bear striking resemblances to those of *Cystopteris*. *Acrophorus* appears to form a link between the more advanced of the Woodsioideae and *Dryopteris*.

The writer wishes to express sincere appreciation for the guidance and help of Professor ALMA G. STOKEY, who collected the material and under whose direction the work was carried out.

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LITERATURE CITED

1. BOWER, F. O., Studies in the phylogeny of the Filicales. II. *Lophosoria*, and its relation to the Cyatheoideae and other ferns. Ann. Bot. **26**: 269–320. 1912.
2. ———, The ferns. Vol. III. Cambridge. 1928.
3. CHRISTENSEN, CARL, Index Filicum. Hafniae. 1906.
4. ———, Filiciniae. In Manual of pteridology. Ed. FR. VERDOORN. The Hague. 1938.
5. DAVIE, R. C., The structure and affinities of *Peranema* and *Diacalpe*. Ann. Bot. **26**: 245–266. 1912.
6. ———, The development of the sorus and sporangium and the prothallus of *Peranema cyatheoides*. Ann. Bot. **30**: 101–109. 1916.
7. DOPP, WALTER, Untersuchungen über die Entwicklung von Prothallien einheimischer Polypodiaceae. Jena. 1927.
8. GOEBEL, K., Organographie der Pflanzen. III. Vol. II. Jena. 1930.
9. HANNIG, E., Über das Vorkommen von Perisporien bei den Filicinen nebst Bemerkungen über die systematische Bedeutungen derselben. Flora **103**: 321–344. 1911.
10. PALSER, BARBARA, and BARRICK, VIRGINIA F., Anatomy and sorus development of *Cystopteris bulbifera*. BOT. GAZ. **103**: 168–176. 1941.
11. SCHLUMBERGER, OTTO, Familienmerkmale der Cyatheaceen und Polypodiaceen und die Beziehungen der Gattung *Woodsia* und verwandter Arten zu beiden Familien. Flora **102**: 383–414. 1911.

ANATOMICAL STUDIES OF THE ROOTS OF JUVENILE LODGEPOLE PINE

RICHARD J. PRESTON, JR.

(WITH EIGHT FIGURES)

Introduction

The roots of forest trees, because of the difficulty and time involved in excavation, have received relatively little attention and study as compared with the above-ground parts. To a great extent the benefits which silvicultural operations have upon a forest result from changes brought about in the root systems, and a more thorough knowledge of the roots will place cutting, thinning, and planting practices on a sounder basis. This investigation was carried out in conjunction with an ecological study concerning the growth and development of root systems (10), in the hope that results might be attained which could be applied to the management of lodgepole pine.

METHODS.—The root systems of 105 specimens of lodgepole pine, *Pinus contorta* var. *latifolia* Engelm., which ranged from 1 to 15 years in age, were excavated during the summers of 1939 and 1940. These excavations were made on two different sites of lodgepole pine in the vicinity of the summer forestry camp of Colorado State College, at Pingree Park, Colorado. The roots were removed by using a pressure sprayer adjusted so as to eject a very fine stream of water. By this manner the root system could be uncovered with so slight damage that it was possible to count the number of mycorrhizae with considerable accuracy.

Anatomical studies of the different orders of roots from freshly excavated seedlings were made. Different techniques were used for the woody and nonwoody portions. The techniques, preserving fluids, and staining schedules used were those outlined by SASS (11).

The growing tips and mycorrhizae were killed and preserved in the Allen-Bouin type 2 formula. After dehydration, these nonwoody structures were infiltrated and imbedded in paraffin, mounted on wooden blocks, and sectioned at approximately 10 μ. They were stained with safranin and fast green. The woody parts were killed and preserved in formula FAA. This material, after dehydration, was infiltrated and imbedded in celloidin, mounted on wooden blocks, sectioned at about 15 μ, and stained with safranin and fast green.

Investigation

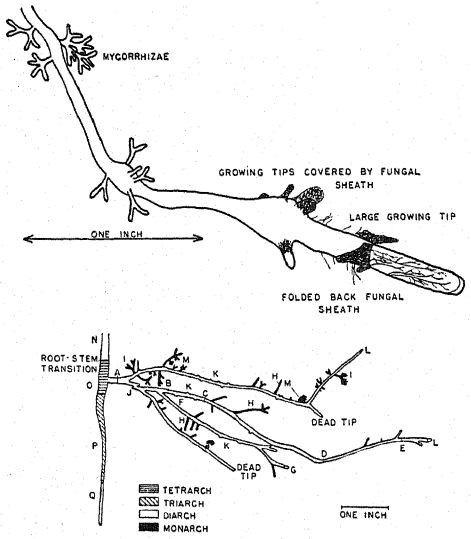
The root system of lodgepole pine can be classified into three main types, which differ in structure and development. These types are the radicle, primary, or taproot; the lateral long-roots; and the lateral short-roots. Lateral long-roots make up the more or less permanent lateral root system. Lateral short-roots differ structurally from long-roots and are temporary structures; in lodgepole pine they normally develop into mycorrhizae.

TAPROOT

The radicle normally developed into a rather deep taproot, which persisted throughout the life of the tree. If

through injury or other cause the taproot did not develop normally, its function was usually assumed by a lateral root, which grew vertically downward to a depth approximately that which the taproot would have attained.

During periods of active elongation, the growing tip became conspicuous, being clear white and on some roots exceeding 2 inches in length (fig. 1). At these periods there was a well-developed root cap of oblong-shaped, thin-walled cells which had their long axes parallel to the axis of the root. The actively growing tips were commonly much thicker than the older portion of the roots, often having a diameter three times as great as the latter (fig. 1), and frequently attaining a diameter of $\frac{1}{8}$ inch. This thickened condition continued back for several inches on fast growing roots, until cork formation resulted in the shedding of cortical cells.



FIGS. 1, 2.—Fig. 1 (above), sketch of root excavated April 13, 1940. Fig. 2 (below), portion of 2-year-old root system indicating stelar structure. Letters indicate points where sections were taken: *A*, *B*, *C*, *D*, *E*, *F*, lateral long-roots; *G*, large growing tip; *H*, long mycorrhiza; *I*, normal mycorrhiza; *J*, *K*, healings; *L*, small growing tip; *M*, ball mycorrhiza; *N*, stem immediately above root-stem transition; *O*, taproots (several) 0.5–1 inch deep; *P*, taproots (several) 1.5–2 inches deep; *Q*, taproots (several) 2.5–3 inches deep.

During periods of dormancy or semidormancy the root cap consisted of only a few cells, and the entire tip was often covered by a typical mycorrhizal sheath. When growth was renewed the tip burst through this sheath and again developed a large root cap (fig. 1).

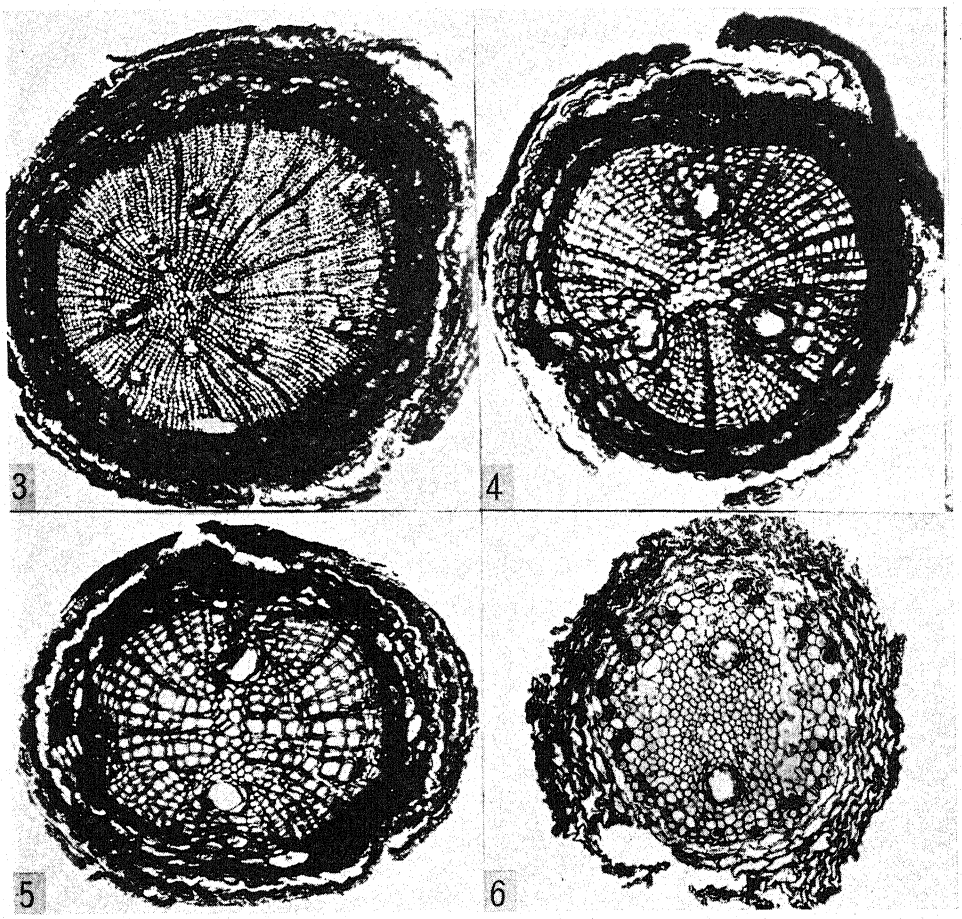
Root hairs developed a short distance behind the tip. According to LAING (5) and HATCH and DOAK (3), these are not epidermal but arise from the second or third layer of cortical cells. They disappeared shortly after the underlying cells became suberized. All actively growing taproots and long-roots had some root hairs, although never the abundant mat typical when grown on moist blotting paper.

Sections of four 2-year-old trees (fig. 2) were taken at distances from the root-stem transition of 0.5–1 inch, 1.5–2 inches, and 2.5–3 inches (hand-sectioning indicated that no structural changes occurred at greater distances). Stained sections through growing tips were likewise studied. No noteworthy differences from typical root development were noted in tissues outside the stele (2). The cortex, consisting of thin-walled, rather isodiametric cells with large intercellular spaces, varied from about four to seven layers in width. The endodermis, consisting of rectangular cells with the long axis arranged tangentially, was distinct.

The number of groups of primary xylem which developed was characteristic. The primary root (figs. 2, 3) was tetrarch for a distance of 0.5–1 inch immediately below the root-stem transition. From this distance to an average of 2.25 inches below it was triarch (fig. 4). Below 2.25 inches and for the remainder

of its development it was diarch (fig. 5). HATCH and DOAK (3) state that radicles are polyarch in *Pinus*, and ALDRICH-BLAKE (1) reports that most taproots are tetrarch throughout their length.

points of protoxylem matured at either side of each resin duct, and as differentiation continued centripetally a Y-shaped group of such cells developed on the side adjacent to each duct. These were con-



FIGS. 3–6.—Cross-sections: Fig. 3, taproot 0.5–1 inch below root-stem transition, tetrarch stele. Fig. 4, taproot 1.5–2 inches below root-stem transition, triarch stele. Fig. 5, taproot 2.5–3 inches below root-stem transition, diarch stele. Fig. 6, growing tip of lateral root, early diarch development.

The primary resin canals were differentiated very early in the development of the tissues (fig. 6) and were clearly evident before the endodermis became distinct. The epithelium of the resin ducts was thin-walled and surrounded by a second row of parenchyma. Two

nected by the differentiation of a bar across the center of the root. Eventually the remaining immature cells in the central portion of the root became differentiated into metaxylem (fig. 5).

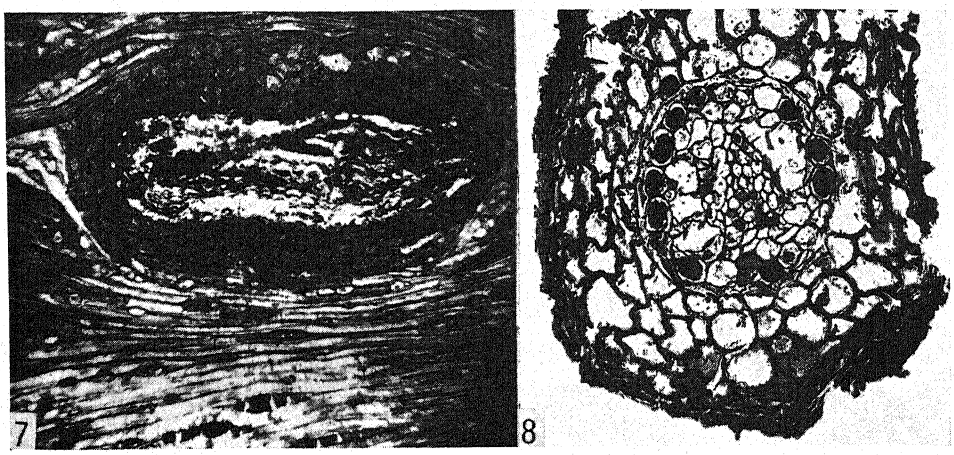
The majority of large secondary roots arose in the region immediately below

the root-stem transition. LIESE (6) concluded that in any particular species the greater the number of primary xylem bundles, the greater would be the abundance of rootlets and therefore the greater the potential absorptive surface of the juvenile root system.

LATERAL LONG-ROOTS

In all cases the lateral long-roots were diarch. There was no apparent difference in their structure from that of the diarch condition of the primary root. No differences were noted in six different lateral roots (fig. 2). HATCH and DOAK (3) state that in *Pinus* lateral long-roots are diarch in their proximal portion. These they term mother roots. More distally, however, they found many to be triarch and applied to these the term pioneer roots. ALDRICH-BLAKE (1) found this development of pioneer roots in Corsican pine but not in Scotch pine. In lodgepole pine lateral long-roots were diarch throughout.

Many diarch lateral roots abort before or during emergence from the parent root, or while still very short. Typical lateral roots show numerous small protuberances (fig. 2) which are either abortive roots or points where roots have died back to the parent root. Sections through these knobs showed that they had developed layers of cork cells over the portions exposed beyond the parent root. Other sections determined the type of healing that occurred when a root died back to a living root (fig. 7). In all cases cork cells were found separating the living from the dead tissue. VATER (12) states that when parts of the roots die off and disappear there is a periderm formed between the living and nonfunctioning portions.



FIGS, 7, 8.—Fig. 7, section through healing on lateral root, formation of periderm. Fig. 8, section through mycorrhizal short-root; monarch structure.

No diarch roots which had been attacked by a fungus and converted into a mycorrhiza (3) were found. HATCH (4) states that these are not common. Monarch short-roots were found which had grown to considerable length before being infected.

LATERAL SHORT-ROOTS

Lateral short-roots differ in several respects from the taproot and lateral long-roots. They are monarch (fig. 8), branch

dichotomously, and have no root caps. HATCH (4) states that mycrotrophy is the normal destiny of all short-roots, that they do not grow much in length, even if uninfected, and that they represent over 95 per cent of all roots in pine. These statements appear valid for lodgepole pine, as nearly all observed short-roots developed into typical ectotrophic mycorrhizae (10).

The mycorrhizal short-roots were not strictly annual structures. Roots excavated at the beginning of the growing season on April 13, 1940 (fig. 1), showed several mycorrhizae which were active, as evidenced by the white tips which had burst or split the fungal sheath. As these had developed the preceding year, this renewed activity rather definitely indicated that these particular roots were not annual. Further, no dead mycorrhizae were found on some vigorous roots several years old. This evidence is at variance with the conclusions of MCDOUGALL (9), MASUI (8), and ALDRICH-BLAKE (1), who state that mycorrhizae are annual. MCDOUGALL also concluded that the fungal sheath inhibited further growth of the roots converted into mycorrhizae, although MASUI (7) demonstrated that this was not necessarily the case, and that it was not uncommon for a mycorrhizal root to continue growth through the fungal sheath at the beginning of the growing season.

Summary

1. Studies of the root systems of juvenile lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.) were made, excavations being carried out on two sites in Colorado. The entire root systems of 105 trees, ranging in age from 1 to 15 years, were excavated with a pressure sprayer.

2. Three main root types were recognized on the basis of structure and growth: the radicle, primary, or taproot; the lateral long-roots; and the lateral short-roots. Structural differences appeared in the stele. The taproot was found to be tetrach in its proximal portion, but more distally triarch and shortly thereafter diarch; all lateral long-roots were diarch throughout; and lateral short-roots were all monarch.

3. The taproot and lateral long-roots developed a typical root cap and root hairs and were capable of extensive growth. The lateral short-roots were not permanent structures, were restricted in growth, and almost invariably formed typical ectotrophic mycorrhizae.

4. The former place of attachment of roots which had died and disappeared, as well as the tips of aborted long-roots, developed a typical periderm which separated the living from the dead cells.

5. During the period of dormancy, mycorrhizal sheaths were found over the tips of lateral long-roots. Upon resumption of growth the tips burst through this sheath. No cases were noted where these long-roots had their growth permanently arrested and were converted into typical ectotrophic mycorrhizae by the fungus.

6. Mycorrhizae did not appear to be strictly annual. Several instances were noted where mycorrhizae had achieved renewed growth at the beginning of the growing season by bursting through the fungal sheath. Active mycorrhizae were also found on roots which were more than one year old.

The writer expresses his appreciation for the helpful assistance received from Dr. A. O. SIMONDS of Colorado State College during the course of this study.

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LITERATURE CITED

1. ALDRICH-BLAKE, R. N., Plasticity of root system of Corsican pine in early life. Oxford Forestry Memoirs. No. 12. 1930.
2. EAMES, A. J., and MCDANIELS, L. H., Introduction to plant anatomy. McGraw-Hill, New York. 1925.
3. HATCH, A. B., and DOAK, K. D., Mycorrhizal and other features of the root system of pine. Jour. Arnold Arbor. 14:85–98. 1933.
4. HATCH, A. B., The physical basis of myrotrophy in *Pinus*. Black Rock Forest Bull. Cornwall-on-the-Hudson, New York. 6:1–168. 1937.
5. LAING, E. V., Studies on tree roots. Forestry Comm. Bull. (Gr. Brit.) 13:3–73. 1932.
6. LIESE, J., Root system of pine. Zeitsch. Forst Jagdwesen. Vol. 3. Berlin. 1926. (As quoted by LAING, 1932.)
7. MAUSUI, K., On the renewed growth of the mycorrhizal root. Mem. Coll. Sci. Kyoto Imperial Univ. Series B. Vol. II, No. 1. 1926.
8. ———, A study of the ectotrophic mycorrhizae of woody plants. Mem. Coll. Sci. Kyoto Imperial Univ. Series B. Vol. III, No. 2. 1927.
9. MCDOUGALL, W. B., Mycorrhizae of coniferous trees. Jour. Forestry 20:No. 3. 1922.
10. PRESTON, R. J., The growth and development of the root system of juvenile lodgepole pine. Ecol. Monog. 12:449–468. 1942.
11. SASS, J. E., Elements of botanical microtechnique. McGraw-Hill, New York. 1940.
12. VATER, H., Die Bewurzelung der Kiefer, Fichte und Buche. Thar. Forstl. Jahrb. 78:65. U.S. Forest Service Transl. 301. 1927.

THE CHLOROPHYLL-PROTEIN COMPLEX. II. SPECIES RELATIONSHIPS IN CERTAIN LEGUMES AS SHOWN BY ELECTRIC MOBILITY CURVES[1]

LAURENCE S. MOYER AND MYER M. FISHMAN

(WITH FOUR FIGURES)

Introduction

Investigations of the specificity of plant proteins, either by means of serological, chemical, or physico-chemical methods, have usually had to deal with mixed systems. MEZ and colleagues (7), in establishing the "*phytoserologische Stammbaum*," generally used seed extracts for immunization. Quantity as well as quality could influence results obtained with these complex protein extracts and possibly lead to false conclusions. Thus absence of a cross reaction might simply indicate that one of the constituents was present at too low a concentration, rather than that an actual difference in quality was present. This complication has been recognized by MORITZ (8), who has developed anaphylactic methods for separation of the various components of the compound antigens; these permit a more complete analysis of serological relationship (9).

Physico-chemical as well as serological methods can be used for the characterization of proteins. Proteins adsorb either H^+ or OH^- and are therefore true amphoteric electrolytes. It has been shown (1) that when the sum of all the positive and negative charges on the surface of protein, over a time average, is equal to zero, its electrophoretic mobility is zero, and the protein or protein surface is isoelectric. Most proteins are isoelectric at acidities somewhat greater than neutral solutions, such that, at values of pH near neutrality, they are negatively charged. But as the pH is decreased, the electrophoretic mobility and net charge likewise decrease, becoming zero at the isoelectric point; below this point the surfaces become increasingly positive (1). The discovery (1) that the electrophoretic mobility of microscopic particles coated with adsorbed films of proteins is nearly or completely identical with that of dissolved protein molecules themselves made possible the investigation of native plant proteins by observation of the electrophoretic behavior of latex particles which are naturally coated with protein films or other ampholytes.

In the investigation of phylogenetic relationships in species of *Euphorbia* and *Asclepias* by the electrophoresis of their latex particles (10–12, 14, 15, 16), complexities similar to those discussed by MORITZ were frequently encountered. Although it was possible to characterize each species by its own electrophoretic mobility curve and isoelectric point, and to group these curves into classes of similar shape, which agreed with taxonomic relationships, nevertheless many species yielded complex curves. These suggested that not only proteins but also other substances with low isoelectric points, possibly sterols, were naturally present in the stabilizing film on the latex particle surface. Indeed, few of the curves had the smooth shape of electro-

[1] This work was materially aided by a grant from the funds of the Graduate School of the University of Minnesota.

phoretic mobility-pH curves of individual proteins (1). This diversity in the surface properties of the various latex particles was substantiated by an investigation of their wetting properties (10). Although phylogenetic relationship was clearly indicated by the results, it was realized at the time that changes in the relative amounts of the proteins and other ampholytes forming the surface films probably had as much to do with changes from species to species as had actual changes in the constitution of the proteins themselves.

Indeed, BONDY and FREUNDLICH (3) showed that the electric mobility curve for the latex of *Hevea brasiliensis* had a complex shape, closely similar to certain curves of latices from the Esulae section of *Euphorbia*, and that this was due to the interaction of two proteins which they isolated. In the pure state either protein exhibited a smooth electrophoretic curve, but when mixed in the presence of microscopic quartz particles, these became coated with a complex film and yielded a complicated curve very similar to that of the natural latex particles.

In view of these complications, it seemed desirable to compare the electrophoretic properties of a similar protein system occurring in a number of species. It would obviously not be significant to compare proteins in various species unless these were alike in function or origin. Inasmuch as it has been possible to isolate the chlorophyll-protein complex in a highly purified form from leaves of bean plants (4), preparation of the corresponding complex from a number of other legumes has been attempted. Since the chlorophyll-protein complex is a system with the same physiological function in these various species, with identical chlorophyll pigments, it seemed clear that the various complexes are legitimately comparable. LUBIMENKO (6) had observed that suspensions of the green material from different species of plants showed pronounced differences in properties. Some leaves gave clear, stable suspensions; some gave clear solutions which were unstable; others appeared to consist of chlorophyll tied to an insoluble protein. Because of the ready availability of young plants from various genera of the Leguminosae, attention was confined to this family for the present.

The literature on the electric mobilities and isoelectric points of other chromoproteins, from various animal species, has already been discussed (1). Some of the data were obtained at a single value of pH. Since such comparisons can lead to erroneous conclusions unless made over a wide range of pH (10), it was decided to determine the electric mobilities of the complex over the range of the acetate buffer system between pH 3.2 and 6.0.

Methods

Leaves from vigorously growing plants were ground in a mortar at 0° C. in the presence of M/100 phosphate buffer at pH 7.3–7.5. The resultant suspensions of the complex were purified by repeated precipitation with ammonium sulphate by the procedure of FISHMAN and MOYER (4). With some species the concentration of the ammonium sulphate was altered somewhat to produce a more clear-cut separation of the complex from the accompanying impurities. All preparations were dialyzed against M/100 phosphate buffers at 0° C. Since no preservatives were used, the electrophoretic measurements were made as soon as possible after purification. The electrophoretic techniques have been fully described elsewhere (1, 4). All measure-

ments were made at 25° C. in acetate buffers at an ionic strength of 0.02.

Results

The taxonomy in this paper follows that of BAILEY (2) and HEGI (5). All species investigated belonged to the section Papilionatae. With two exceptions the chlorophyll-protein complexes isolated from the various species were identical in general appearance. The dark green suspensions, although opaque, were quite stable. Attempts to prepare the complex from leaves of *Arachis hypogaea* produced a dark green suspension that blackened rapidly and became unstable. It seems likely that this anomalous behavior was due to contaminants not present in the other species. The complex from leaves of *Lupinus albus* became flocculated in the phosphate buffer and could not be resuspended. It is possible that its isoelectric point is so high that it is unstable at pH 7.3. These two genera belong to different tribes from those to which the rest of the genera belong: *Arachis* to the Coronilleae and *Lupinus* to the Genisteae. The other genera belong to (*a*) the Phaseoleae, (*b*) the Vicieae, or (*c*) the Trifolieae.

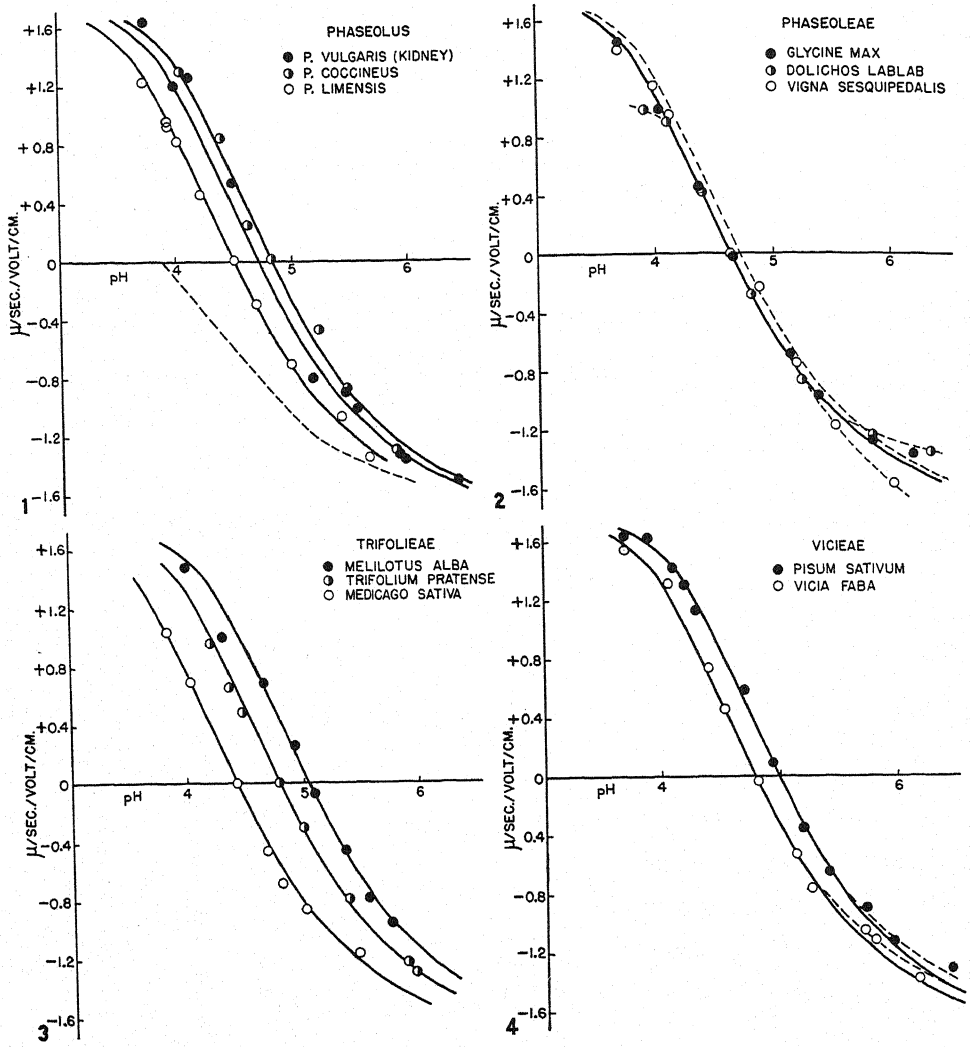
The electrophoretic curves were all regular in shape and characteristic in each case of a single protein. Each species could be characterized by a definite behavior. It soon became apparent that a single smooth curve would fit the data for any species, irrespective of the tribe to which it belonged. This fit resulted in each case when the curve was adjusted to the observed isoelectric point by shifting it to the left or right along the abscissa. Thus in figure 1, which represents the electric mobility-pH behavior of the chlorophyll-protein complex from species of *Phaseolus*, the central continuous curve (isoelectric at pH 4.70), which was drawn to fit the original data for navy bean (4), also describes the electrophoretic properties of the complex from *P. coccineus* and *P. limensis* when shifted to their respective isoelectric points. In fact, the original curve for navy bean fits the data for the closely related kidney bean without adjustment.

The curve of FISHMAN and MOYER for *Aspidistra elatior* is also plotted as a dashed line in figure 1. Since acid denaturation caused considerable shift and resultant discontinuity in the curve on the acid side of the isoelectric point, the curve has been ended at pH 3.9. Its slope and isoelectric point are completely different from the results on legumes.

In figure 2 are shown the results for *Vigna sesquipedalis*, *Dolichos lablab*, and *Glycine max*. In general, these fall along the same curve as in figure 1. Slight possible deviations have been indicated as dashed lines in the case of *Vigna* and *Dolichos*, but *Glycine* essentially follows the continuous curve. The complex from all three species appears to have the same isoelectric point. For comparison, a parallel curve (dashed) has been drawn to indicate the position of the data for *P. vulgaris*.

Figure 3 depicts the behavior of certain members of the Trifolieae: *Medicago sativa*, *Trifolium pratense*, and *Melilotus alba*. These results follow a course similar to the others.

The electric mobilities of the complex from *Vicia faba* and *Pisum sativum*, two genera of the Vicieae, are shown in figure 4. Here also the same curve has been superimposed on each set of data with equal agreement. A slight deviation appears to occur in each case. If significant, this common change in slope may serve to set the Vicieae apart from the other groups. Before this can be substantiated, more basic values of pH must be investi-



FIGS. 1–4.—Electrophoretic mobility–pH curves of chlorophyll-protein complex, in M/50 acetate buffer, at $\mu = 0.02$ and 25° C.: Fig. 1, kidney bean (solid circle), *P. coccineus* (half-solid circle), and *P. limensis* (open circle); smooth curves taken from data for navy bean, dashed curve from *Aspidistra elatior* (drawn for comparison). Fig. 2, *Glycine max* (solid circle), *Dolichos lablab* (half-solid circle), and *Vigna sesquipedalis* (open circle); smooth curve taken from data for navy bean (deviations indicated by dashed lines); parallel (dashed) curve is that of navy bean. Fig. 3, *Melilotus alba* (solid circle), *Trifolium pratense* (half-solid circle), and *Medicago sativa* (open circle); smooth curves taken from data for navy bean. Fig. 4, *Pisum sativum* (solid circle) and *Vicia faba* (open circle); smooth curves taken from data for navy bean (deviations indicated by dashed lines).

gated. To extend the curves to higher values of pH necessitates a change in the buffer system. Attempts to continue the curve for *P. vulgaris* by use of phosphate buffers (at the same ionic strength) led to an anomalous displacement in the curve, similar to that observed with rabbit serum proteins under comparable conditions (17). In view of this complication, the investigation of more basic regions has been deferred.

TABLE 1

ISOELECTRIC POINTS OF CHLOROPHYLL-PROTEIN COMPLEX FROM VARIOUS LEGUMES, AT 25° C. IN M/50 ACETATE BUFFERS

Species	Isoelectric point pH
Phaseoleae	
Phaseolus vulgaris var. navy bean	4.70
P. vulgaris var. kidney bean	4.70
P. limensis var. bush lima	4.50
P. coccineus	4.82
Vigna sesquipedalis	4.62
Dolichos lablab	4.62
Glycine max	4.62
Trifolieae	
Medicago sativa	4.42
Trifolium pratense	4.78
Melilotus alba	5.06
Viceae	
Vicia faba	4.78
Pisum sativum	4.96

TABLE 2

DATA FOR CONSTRUCTION OF TYPICAL CURVE FOR CHLOROPHYLL-PROTEIN COMPLEX FROM BEAN IN M/50 ACETATE BUFFERS AT 25° C.

pH	Electric mobility (μ/sec.)	pH	Electric mobility (μ/sec.)
3.5	+1.65	5.1	−0.59
3.7	+1.53	5.3	−0.83
3.9	+1.36	5.5	−1.02
4.1	+1.06	5.7	−1.17
4.3	+0.73	5.9	−1.31
4.5	+0.39	6.1	−1.41
4.7	0	6.3	−1.49
4.9	−0.30	6.5	−1.57

A list of isoelectric points has been assembled in table 1. Data for the construction of the characteristic smooth curve are given in table 2.

Discussion

At the start of these investigations, the close agreement found by SMITH (18) in the analyses of the chlorophyll-protein complexes from *Aspidistra* and *Spinacia* suggested that no essential differences would be found among genera. The marked divergences between the electrophoretic properties of the *Aspidistra* and bean preparations, however, indicated that clear differences did exist between widely separated genera (4). Within a homogeneous section like the Papilionatae there occurs less variation in physical characteristics of the complexes than exists between families and larger categories. Indeed, it is possible that in the Papilionatae the photosynthetic pigments are associated with essentially the same protein, which becomes only slightly modified from species to species.

Except in those cases in which divergences at the ends of the ideal curve have been suggested in the figures, the agreement of the data with the shifted curve is usually well within the limits of experimental error. As indicated, slight modifications do occur, but the general picture is essentially one of a single smooth curve, shifted up or down the pH scale but not changed in other features. A somewhat similar case was found with the latices from species of *Asclepias* (14). In that work it was suggested that the data could be fitted by a single curve, but that, owing to the narrow range of the buffer system, various parts of the whole curve fitted the results for each of the species investigated. It was as though the same general curve shape was being

retained, even though the isoelectric point changed from species to species.

Summary

1. The chlorophyll-protein complex has been prepared from leaves of the following legumes: *Phaseolus vulgaris*, *P. limensis*, *P. coccineus*, *Vigna sesquipedalis*, *Dolichos lablab*, *Glycine max*, *Medicago sativa*, *Trifolium pratense*, *Melilotus alba*, *Vicia faba*, and *Pisum sativum*.

2. Electrophoretic mobility-pH curves of the complex from these species show close relationship but differ completely from the curve for *Aspidistra*.

3. The various sets of data from these legumes could be described by a single smooth curve shifted up or down the pH scale to pass through each isoelectric point. In some cases minor deviations were noticed, but in general the agreement was good.

4. It is suggested that the complex from each species is characterized by essentially the same protein, slightly altered in certain respects from the rest but retaining enough of its original character to show relationship.

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LITERATURE CITED

1. ABRAMSON, H. A., MOYER, L. S., and GORIN, M. H., Electrophoresis of proteins. Reinhold Publ., New York. 1942.
2. BAILEY, L. H., Manual of cultivated plants. New York. 1924.
3. BONDY, C., and FREUNDLICH, H., The proteins in preserved *Hevea* latex. Compt. rend. Trav. Lab. Carlsberg **22**:89–98. 1937.
4. FISHMAN, M., and MOYER, L. S., The chlorophyll-protein complex. I. Electrophoretic properties and isoelectric point. Jour. Gen. Physiol. **25**:755–764. 1942.
5. HEGI, G., Illustrierte Flora von Mittel-Europa. IV: 3. Munich. 1926.
6. LUBIMENKO, V. N., Les pigments des plastes et leur transformation dans les tissus vivants de la plante. Rev. Gén. Bot. **39**:619–637. 1927.
7. MEZ, C., and ZIEGENSPECK, H., Der Königsberger serodiagnostische Stammbaum. Bot. Arch. **13**:483–485. 1926.
8. MORITZ, O., Prinzipien und Beispiele der Anwendung phytoserologischer Methodik. Planta **15**:647–697. 1932.
9. MORITZ, O., and VOM BERG, H., Serologische Studien über das Linswickenproblem. Biol. Zentralbl. **51**:290–309. 1931.
10. MOYER, L. S., Species relationships in *Euphorbia* as shown by the electrophoresis of latex. Amer. Jour. Bot. **21**:293–313. 1934.
11. ———, Electrophoresis of latex and chromosome numbers of poinsettias. BOT. GAZ. **95**: 678–685. 1934.
12. ———, The constancy of latex isoelectric points. Protoplasma **21**:588–593. 1934.
13. ———, On the surface composition of certain latex particles. Amer. Jour. Bot. **22**:609–627. 1935.
14. ———, Chromosome numbers and electrophoresis of latex in *Asclepias*. BOT. GAZ. **97**: 860–865. 1936.
15. ———, Recent advances in the physiology of latex. Bot. Review **3**:522–544. 1937.
16. ———, The use of electrophoresis in the elucidation of biological problems. Trans. Faraday Soc. **36**:248–256. 1940.
17. MOYER, L. S., and MOYER, E. Z., Electrokinetic aspects of surface chemistry. VII. The electrophoretic behavior of microscopic particles in the presence of horse, human, or rabbit serum. Jour. Biol. Chem. **132**:373–386. 1940.
18. SMITH, E. L., The chlorophyll-protein compound of the green leaf. Jour. Gen. Physiol. **24**:565–582. 1941.

EFFECTS OF VITAMINS ON GERMINATION AND GROWTH OF ORCHIDS[1]

G. R. NOGGLE AND F. L. WYND

Introduction

It is held by many students that the need for the symbiotic relationship between epiphytic orchids and certain fungi is eliminated if an appropriate supply of soluble carbohydrate be present in the substrate. The observation of many hundreds of orchid cultures, however, grown at the Missouri Botanical Garden, under many conditions of nutrition, shows that rarely do asymbiotic cultures thrive as well as those containing the appropriate symbiotic fungus. This has led to the assumption that the fungal symbiont was important for some reason in addition to that of hydrolyzing insoluble carbohydrates in the substrate so that they might be absorbed by its host.

The relation of the fungus to the acidity of the medium has been discussed by KNUDSON (13). The possible importance of the fungus in the nitrogen metabolism of the host has been suggested by others. TERNETZ (31) found that the fungal symbionts isolated from the roots of five species of the Ericaceae were able to fix atmospheric nitrogen in artificial cultures. DUGGAR and DAVIS (6) and RAYNER (22) also reported that nitrogen could be fixed by symbiotic fungi. The conclusions of RAYNER have been criticized on the basis that the amount of nitrogen fixed was so small that it was within the range of experimental error. WOLFF (34) believed that the endophyte of the orchid *Neottia* could fix atmospheric nitrogen, which was subsequently available to the host.

Recent investigations have emphasized the importance of certain vitamins and other growth factors for development of plants. It seems reasonable to suppose that the fungal symbiont might exert a favorable effect on the development of orchid seedlings by supplying some of these factors, in addition to hydrolyzing insoluble carbohydrates, and thereby account for their more rapid growth when cultured symbiotically.

An example of microörganisms supplying a growth factor to higher plants was reported by MCBURNEY, BOLLEN, and WILLIAMS (17), who found that the nodule-forming bacterium owes part of its stimulating effect to the pantothenic acid which was supplied to the host. MOCKERIDGE (18) showed that substances produced by bacteria increased the growth of *Lemna*. WILLIAMS and ROHRMAN (32) reported that the liverwort *Ricciocarpus* responded by marked increase in growth when pantothenic acid was added to the culture medium. ROBBINS (24) and MÜLLER and SCHOPFER (19) have suggested that symbiosis and parasitism might be intimately related to the ability of one member of the association to supply growth factors to the other.

BURGEFF (4) pointed out that certain physiologically highly specialized orchids of the *Vanda* group always developed slowly in the absence of their natural symbionts. The dead fungus was found to be just as effective as the living hyphae, because of the presence of a growth factor resembling "bios II," although its chemical nature was otherwise unknown.

More recently, WITHNER (33) cultured *Cattleya octave doin* × *C. mossiae* and *Epidendrum tempense* on media which contained various growth factors. He found that no effect was exerted by vitamin B_1, vitamin B_2, vitamin B_6, vitamin C, niacin, biotin, pantothenic acid, and

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inositol. On the other hand, POLLACCI and BERGANISCHI (20) found that the presence of vitamin C in the medium resulted in increased germination and accelerated the growth of *Cattleya labiata autumnalis* and *Oncidium pulvinatum*.

The necessity of an external supply of various growth factors for higher plants has been reported. The importance of vitamin B_1 has been shown (14, 3, 27). The influence of vitamin C in germination and growth has also been recorded (9, 3, 10). The importance of vitamin B_2 in the nutrient medium for the growth of higher plants has not yet been conclusively demonstrated. The present study sought additional information on the effect of certain vitamins on the germination of the seeds and on the growth of orchids.

Investigation

EXPERIMENT I

A mature seedpod of the hybrid, *Cattleya trianae* var. *mooreana* × *C. schroederae*, was obtained through the generosity of DR. GEORGE T. MOORE, Director of the Missouri Botanical Garden. It had been picked in January, 1940, and had been kept in a refrigerator at 7° C. until the seeds were sown, February 5, 1941. The nutrient solution was the type I, R_1S_2, described by LIVINGSTON (16), which had produced excellent germination and growth during earlier experiments (35, 36). In addition to the six major nutrient ions, iron, manganese, and boron were added as formerly, and the pH was adjusted to 5.0. The carbohydrate was supplied as 1.5 per cent maltose. Solidification was obtained by 2 per cent Difco agar. (The carbohydrate was a technical grade of maltose, manufactured by Daigger and Company. Its light brown color and gummy texture indicated that the material had not been highly refined.) Ten flasks were maintained as a control series; ten received 0.1 mg. per liter of thiamin hydrochloride, ten received 0.01 mg., and ten received 0.005 mg. per liter of this vitamin.

Good germination and growth occurred when these nutrient media were used. The addition of thiamin hydrochloride was without effect. On October 7, 1941, 8 months later, the seedlings were transplanted by means of an inoculating needle to flasks of media freshly prepared as before, except that Pfansteil's reagent maltose was substituted for the older, less refined sugar. Some of these cultures received 10 mg. per liter of ascorbic acid (vitamin C), others received 1 mg. per liter, while others served as control cultures. Although sixty flasks of transplanted seedlings composed this series, in every flask the seedlings turned yellow in a few weeks and finally became almost white. Significant growth did not occur, and all seedlings died within 3 months. Tests made on the media showed that the proper acidity had been maintained. There was no effect due to any concentration of ascorbic acid.

EXPERIMENT II

Another pod of *Cattleya* hybrid which showed a remarkably high percentage of fertile seeds was obtained from the Missouri Botanical Gardens. These seeds were inoculated into series of media prepared as in experiment I, except that the thiamin hydrochloride was added in a greater series of concentrations (10.0, 5.0, 1.0, 0.5, 0.1, 0.05, 0.01, and 0.001 mg. per liter). The carbohydrate, unlike that of experiment I, was the highly purified reagent grade of maltose prepared by the Pfansteil Company.

After 6 weeks the protocorms were still so small that they could scarcely be seen with the unaided eye. After several months they had increased materially in size but had become a shapeless structure

of parenchymatous tissue. Normal leaves did not appear, and chlorophyll was absent—or present only in traces. The physical conditions had been ideal, and tests showed that the proper acidity had been maintained. There was no effect from the addition of thiamin hydrochloride.

Experiment III

The results of experiments I and II indicated that the impure technical grade of maltose contained some factor or factors necessary for the germination and growth of orchid seedlings which were not present in the particular sample of purified maltose. It also appeared that neither ascorbic acid nor thiamin hydrochloride could replace these substances. In the meantime, a third experiment had been started, using the seeds from the same pod as those used in experiment II. The media were prepared with the reagent grade of maltose as the carbohydrate. Riboflavin (vitamin B_2), nicotinic acid (P-P factor), pyridoxine hydrochloride (vitamin B_6), and calcium pantothenate (chick antidermatitis factor) were added, each to separate cultures, in concentrations of 10 and 1 mg. per liter. After 14 months the following observations were made:

Control.—These cultures showed practically no germination. Not more than a dozen seeds out of many hundreds formed protocorms, and these remained small, distorted in shape, and without chlorophyll. All were dead within a few months.

Calcium pantothenate.—No significant germination occurred.

Riboflavin.—Very limited germination occurred. The protocorms averaged 0.88 mm. in diameter, and they had developed one to three normally shaped but very small green leaves. Germination was so poor and subsequent growth so limited that the beneficial effect of this vitamin appears doubtful. The seedlings did develop a green color, however, which was not the case for the controls.

Pyridoxine.—Excellent germination occurred, but subsequent development was slow. The protocorms became abnormally large and were frequently distorted in shape. The protocorms averaged 1.12 mm. in diameter. A few had developed one or two rudimentary leaves, although most of them exhibited only one or two undeveloped leaf points. The average height was 2.40 mm. Chlorophyll development was conspicuously less than normal. Perhaps one-half the protocorms developed a pale green color, but the others remained with little or no chlorophyll.

Nicotinic acid.—This series produced excellent germination, and subsequent development was good. The protocorms remained small, and normal green leaves developed. The concentration of 1 mg. per liter produced the most rapid growth of the seedlings. In this concentration the protocorms averaged 0.86 mm. in diameter and the seedlings averaged 4.40 mm. in height. The average number of well-developed leaves was 3.16. This was the only medium in which the seedlings developed aerial roots, some of which attained a length of 6 mm. The concentration of 10 mg. per liter produced protocorms which averaged 0.75 mm. in diameter and seedlings which averaged 3.44 mm. in height, while the number of normal leaves per plant averaged 2.40.

Although the seedlings in these cultures appeared to have normal shape and color, their growth was far less than that of normal seedlings of an equal age. Under the most favorable conditions, orchid seedlings should be about $\frac{1}{4}$ inch high in 4 months. At 10–12 months they should be large enough to be transplanted. Apparently there are other factors or

combinations of factors necessary for the normal growth of orchids besides those described here.

Instances in which erratic results in various types of culture experiments were traceable to biologically active impurities in the reagents are not infrequent. The probability that commercially prepared sugars vary significantly in their freedom from effective growth factors may account for the divergent views of various students concerning the most favorable type of sugar for orchid germination and growth. LA GARDE (15) stated that maltose was the best sugar for the growth of orchids, QUEDNOW (21) found d-glucose the best, while WYND (36) found d-mannose to give the best growth. WITHNER (33) reported that sugar has been shown to contain small amounts of vitamin B_1, vitamin B_6, biotin, and probably other substances.

The carbohydrates are not the only sources which might supply growth factors to cultures as impurities. Agar contains variable amounts of impurities, even when it is highly purified. ROBBINS and WHITE (29) showed that some of these impurities were biologically effective, since a water extract of agar increased the growth rate of excised corn roots. ROBBINS (23) later reported that Difco agar exerted a beneficial effect on the germination of spores, growth, and the formation of gametes and zygotes by *Phycomyces blakesleeanus*. The stimulating effect was believed to be due to unrecognized growth factors. DAY (5) found that *P. blakesleeanus* grew better when Difco or Eimer & Amend agar was added to the medium. Since no thiamin had been added, she concluded that this necessary substance was introduced as an impurity of the agar. ALLISON and HOOVER (1) demonstrated the presence of coenzyme R (= biotin or vitamin H) in agar. HAWKER (11, 12) found biotin to be present in agar. Thiamin has been found in agar by FRIES (7) and FROMAGEOT and TCHANG (8). Unidentified growth substances, called factor Z, were found in agar by ROBBINS (25). ROBBINS (26) and ROBBINS and MA (28) found significant amounts of biotin in some samples of agar as determined by the growth of *Ashbya gossypii*. SCHOPFER and RYTZ (30) found that crude cotton contained thiamin or its intermediate products. DAY (5) found that cheesecloth and filter paper also contained sufficient thiamin to affect the growth of *Phycomyces*.

In view of the ever-present possibility of introducing growth substances into culture media by impure reagents or contact with material used in the laboratory, different workers may not obtain similar results in this field of research unless extremely careful precautions are taken in laboratory procedures.

Summary

1. Seeds of the orchid *Cattleya trianae* var. *mooreana* × *C. schroederae* germinated and produced normal growth in an artificial nutrient medium in which one lot of maltose was used as a source of carbohydrate, but no germination of seeds or growth of seedlings was obtained when a more purified maltose was used.

2. The inability of the orchid seeds to germinate and to develop on the purified maltose was not overcome by the addition of thiamin hydrochloride (vitamin B_1), ascorbic acid (vitamin C), or calcium pantothenate (chick antidermatitis factor).

3. A few seeds germinated and slow development of the seedlings occurred when riboflavin (vitamin B_2) was present in the medium.

4. The presence of pyridoxine (vita-

min B_6) permitted good germination, but subsequent development was poor.

5. Good germination and excellent development of the seedlings occurred when nicotinic acid (P-P factor) was supplied in the nutrient medium.

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LITERATURE CITED

1. ALLISON, F. E., and HOOVER, S. R., An accessory factor for legume nodule bacteria. Jour. Bact. 27:561–581. 1934.
2. BONNER, J., Vitamin B_1 a growth factor for higher plants. Science 85:183–184. 1937.
3. BONNER, J., and AXTMAN, G., The growth of plant embryos *in vitro*. Preliminary experiments on the role of accessory substances. Proc. Nat. Acad. Sci. 23:453–457. 1937.
4. BURGEFF, H., Pflanzliche Avitaminose und ihre Behebung durch Vitaminzufuhr. Ber. deutsch. Bot. Ges. 52:384–390. 1934.
5. DAY, DOROTHY, Thiamin content of agar. Bull. Torr. Bot. Club 69:11–20. 1942.
6. DUGGAR, B. M., and DAVIS, A. R., Studies in the physiology of the fungi. I. Nitrogen fixation. Ann. Missouri Bot. Gard. 3:413–437. 1916.
7. FRIES, NILS, Ueber die Bedeutung von Wuchsstoffen für das Wachstum verschiedener Pilze. Symb. Bot. Upsol. III. 2:1–188. 1938.
8. FROMAGEOT, CLAUDE, and TCHANG, J. L., Sur la synthèse des pigments caroténoïdes par *Rhodotorula sanniei*. Arch. Mikrobiol. 9:434–448. 1938.
9. HAUSEN, SYNNOVE v., Die Rolle des Vitamins C beim Wachstum der höheren Pflänzen. Biochem. Zeit. 288:378–392. 1936.
10. HAVAS, L., Ascorbic acid (vitamin C) and the germination and growth of seedlings. Nature 136:435. 1935.
11. HAWKER, LILIAN H., The effect of certain accessory growth substances on the sporulation of *Melanospora destruens* and of some other fungi. Ann. Bot. 50:699–718. 1936.
12. ———, The nature of the accessory growth factors influencing growth and fruiting of *Melanospora destruens*. Ann. Bot. n.s. 3:657–676. 1939.
13. KNUDSON, L., Physiological study of the symbiotic germination of orchid seeds. BOT. GAZ. 79:345–380. 1925.
14. KÖGL, F., and HAAGEN-SMIT, A., Biotin und Aneurin als Phytohormone. Zeit. physiol. Chem. 243:209–226. 1936.
15. LA GARDE, R. V., Non-symbiotic germination of orchids. Ann. Missouri Bot. Gard. 16:499–514. 1929.
16. LIVINGSTON, B. E., A plan for cooperative research on the salt requirements of representative agricultural plants. Baltimore. 1919.
17. MCBURNEY, C. H., BOLLEN, W. B., and WILLIAMS, R. J., Pantothenic acid and the nodule bacteria-legume symbiosis. Proc. Nat. Acad. Sci. 21:301–304. 1935.
18. MOCKERIDGE, FLORENCE A., The formation of plant growth promoting substances by microörganisms. Ann. Bot. 38:723–734. 1924.
19. MÜLLER, WERNER, and SCHOPFER, W. H., L'action de l'aneurine et de ses constituents sur *Mucor ramanneanus* Möll. Compt. rend. Paris 205:687–689. 1937.
20. POLLACCI, G., and BERGANISCHI, M., (Effect of vitamins on seeds of orchids.) Boll. Soc. Ital. Biol. Sper. 15:326–327. 1940.
21. QUEDNOW, K. G., Beitrage zur Frage der Aufnahme geloster Kohlenstoffverbindungen und andere Pflanzen. Bot. Archiv. 30:51–108. 1930.
22. RAYNER, M. C., Nitrogen fixation in the Ericaceae. BOT. GAZ. 73:226–235. 1922.
23. ROBBINS, W. J., Growth substances in agar. Amer. Jour. Bot. 26:772–778. 1939.
24. ———, Thiamin and plant growth. Science 89:303–307. 1939.
25. ———, Further observations on factor Z. BOT. GAZ. 102:520–535. 1941.
26. ———, Biotin and the growth of *Fusarium avenaceum*. Science 93:437–438. 1941.
27. ROBBINS, W. J., and BARTLEY, MARY, Vitamin B_1 and the growth of excised tomato roots. Science 85:246–247. 1937.
28. ROBBINS, W. J., and MA, ROBERTA, Biotin and the growth of *Fusarium avenaceum*. Bull. Torr. Bot. Club 69:446–462. 1941.
29. ROBBINS, W. J., and WHITE, VIRGINIA B., Limited growth and abnormalities in excised corn root tips. BOT. GAZ. 98:209–242. 1936.
30. SCHOPFER, W. H., and RYTZ, W., JR., La ouate comme source de facteur de croissance de microorganisme. Arch. Mikrobiol. 8:244–248. 1937.
31. TERNETZ, C., Über die Assimilation des atmosphärischen Stickstoff durch Pilze Prings. Jahrb. wiss. Bot. 64:353–408. 1907.
32. WILLIAMS, R., and ROHRMAN, E., Pantothenic acid as a nutrilite for green plants. Plant Physiol. 10:559–563. 1935.
33. WITHNER, C. L., Nutrition experiments with orchid seedlings. Amer. Orchid Soc. Bull. 11:112–114. 1942.
34. WOLFF, J., Observations sur les divers modes de culture des orchidées. Rev. Path. Vég. Ent. Agr. 12:185–200. 1925.
35. WYND, F. L., The sensitivity of orchid seedlings to nutritional ions. Ann. Missouri Bot. Gard. 20:223–237. 1933.
36. ———, Sources of carbohydrate for germination and growth of orchid seeds. Ann. Missouri Bot. Gard. 20:569–581. 1933.

TRANSPLANTATION EXPERIMENTS IN PEAS. III[1]

F. W. WENT

Introduction

Earlier papers (**12, 5**) have shown that a number of specific growth factors can be transported across the graft union of pea seedlings as soon as vascular connections between the graft partners have been established. By choosing the proper combinations, either the growth-factor content of the stock or the growth response of the scion can be studied. In this way a tool is provided for the investigation of growth factors which cannot be extracted as yet. Although this type of experiment has yielded many important results in embryonic development of animals, and also has been shown adaptable to plants, very little work has been published on the graft technique for growth-factor analysis. The most important contribution in this field is that of WETTSTEIN and PIRSCHLE (**11**) concerning a gene-determined factor in *Petunia* transmitted by grafting.

The conditions under which the experiments were carried out are almost identical with those prevailing in the earlier experiments of this series (**12, 5**). From soaking until termination of the experiment, the peas are kept in physiological darkrooms, maintained at 24° C. and 80 per cent humidity. An orange light is kept on for 4–8 hours each day in the rooms, providing enough light for leaf and stipule development (**14**).

Some varieties of pea have to be disinfected before planting by washing them for 1 minute in 90 per cent alcohol, followed by 20 minutes in 0.1 per cent $HgCl_2$, and repeated washing with sterile water. Then they are soaked for 5 hours in water and spread out on moist filter paper in dishes. As soon as the roots have reached a length of 1–2 cm., the peas are transferred singly to 20-cc. wide-neck bottles or glass tubes. The seed coats have to be removed to prevent growth of molds and bacteria. A few drops of water are added, enough to provide for 1 day's growth. Each day the required amount of water is supplied, but not enough to submerge the roots (this would greatly decrease the growth rate of the shoots). Six to 8 days after soaking (or longer for a few slow-growing varieties), the peas are grafted by cutting the stem at a 30° angle and slipping the two cut ends through a short glass tube with approximately the same bore as the stem diameter. From then on, daily measurements are taken to determine maximal growth rates.

The grafted peas are inspected daily, and lateral buds developing on the stock are removed before they can inhibit growth of the scion. Throughout this period the root system is kept moist but never submerged in water.

When growth in length of the main stem of the scion has stopped, lateral buds will develop. These buds soon grow 50 per cent more rapidly than the main shoot. For this reason, growth rates of laterals and main stem cannot be averaged. At the end of the growth period the length of the leaflet, stipule, petiole, and tendril of each successive leaf on the scion is measured. In general, the oldest leaflet is already almost full-grown at the time of grafting, so that it

[1] Report of work carried out with the assistance of Work Projects Administration, Work Project no. 12165, Official Project no. 165-1-07-172.

does not show a clear stock effect; but the dimensions of the younger leaves can be used for calculations of the stock-scion relationship.

The present paper describes some further experiments concerning the technique of grafting, some experiments concerning the nature of the stock-scion relation, and also a few applications of the technique.

shorter than in the regular grafting experiments. The 8-day-old peas were 130–200 mm. long. The 10-day-old plants were 250–300 mm. long—much longer than otherwise used for grafting. The results as presented in table 1 can be summarized as follows:

Six- and 8-day-old peas, within a range of 50–200 mm. length, are equally suitable for grafting, both as stock and as

TABLE 1

EFFECT OF AGE OF ROOT STOCK AND SCION OF ALASKA PEAS AT TIME OF GRAFTING: ON PERCENTAGE OF SUCCESSFUL GRAFTS (EACH GROUP CONSISTING OF ABOUT FIFTEEN), ON MAXIMAL GROWTH RATE OF SCIONS AFTER GRAFTING, ON LENGTH OF SUM OF SECOND AND THIRD INTERNODES OF SCION, ON LEAF, AND ON STIPULE SURFACE OF SCIONS (TAKEN AS MEAN OF SECOND AND THIRD LEAVES)

Stock	Scion											
	Ten days old		Eight days old					Six days old				
	Percentage take	Growth rate (mm./day)	Percentage take	Growth rate (mm./day)	Length of second and third internodes (mm.)	Leaf surface (mm.²)	Stipule surface (mm.²)	Percentage take	Growth rate (mm./day)	Length of second and third internodes (mm.)	Leaf surface (mm.²)	Stipule surface (mm.²)
Ten days old.....	20	19	21	17	75	32	46	67	25	87	19	25
Eight days old.....	21	20	62	32	125	42	48	56	33	139	32	40
Six days old.....	7	.....	69	33	140	58	54	77	31	139	28	36

Experimentation

AGE OF STOCK AND SCION AT TIME OF GRAFTING

It had already been noted that when peas are left too long growing in darkness, the percentage of successful grafts with the seedlings is very low. Alaska peas were soaked and planted in glass bottles at 2-day intervals. When the different groups were 6, 8, and 10 days old, all possible graft combinations among the three groups were made. The 6-day-old plants were 50–100 mm. long—scion. The 10-day-old plants are very poor for grafting. This holds only for peas grown in a darkroom, however, when no assimilation occurs and the cotyledons are becoming exhausted.

TREATMENT OF GRAFT SURFACE WITH HORMONES

In some preliminary experiments, Dr. A. C. SINHA, working in this laboratory, had observed certain effects of the treatment of the cut surface of pea seedlings just prior to grafting. Lanolin, either plain or mixed with various con-

centrations of indoleacetic acid, was injurious and killed the stems adjoining the graft in a few days, without any indication of junction of stock and scion. Dipping the cut ends of stock and scion for a few seconds in aqueous solutions of either indoleacetic and traumatic acids (100 p.p.m.) seemed to improve the taking of the grafts. This has been investigated in greater detail.

Peas, 7–8 days after germination, were grafted on themselves after removal of an intermediate stem piece approximately 50–100 mm. long. Before pressing the cut surfaces together in the glass tube, they were dipped for 1–2 seconds in various solutions. Afterward the growth measurements were taken in the ordinary way. Table 2 shows the results. Just 50 per cent of all grafts (forty per group) were successful. There was no preference for any treatment. The maximal growth rates, however, which were slightly higher than in most experiments, were all within the limits of experimental error. The same can be said of the leaf sizes of the scions and of the time necessary to reach maximal growth rate.

Another experiment indicated some improvement in maximal growth rate and percentage of successful cuttings when the cut ends are treated with a lower concentration (50 p.p.m.) of indoleacetic acid, but these increases do not warrant a change in the experimental procedure of grafting.

An investigation of the correlation between the length of the glass collar around the graft union and the growth of the scion showed that the mean length of the tube in 162 unsuccessful grafts was 7.95 mm., and in 200 successful grafts it was 7.95 mm. (the actual lengths varied between 4 and 12 mm.). In the successful grafts there was a slight preponderance (47 per cent) of the collars of 7 and 8 mm. length, compared with the unsuccessful grafts (only 36 per cent of the collars having a length of 7–8 mm.).

TABLE 2

MAXIMAL GROWTH RATES AND LEAF DEVELOPMENT OF SCIONS OF ALASKA PEAS, GRAFTED ON ALASKA STOCKS AFTER IMMERSION OF CUT SURFACES IN SOLUTIONS. FORTY GRAFTS IN EACH GROUP

Cut ends of stock and scion washed with	Percentage successful grafts	Maximal growth rate		Leaf length (mm.)		
		Rate (mm./day)	Reached after (days)	First leaf	Second leaf	Third leaf
Distilled water	52	46.2	7.5	5.8	6.3	7.0
Thiamin (1 p.p.m.)	45	42.9	7.8	5.6	6.1	7.0
Indoleacetic acid (50 p.p.m.)	45	47.7	8.0	5.2	6.7	6.7
Traumatic acid (100 p.p.m.)	55	46.3	7.6	5.6	6.7	7.0
Thiamin (0.67 p.p.m.), indoleacetic acid (33 p.p.m.), traumatic acid (67 p.p.m.)	52	47.3	7.4	5.8	6.6	7.5

EFFECT OF INTERMEDIATE STEM PIECE ON GROWTH OF SCION

ROBERTS (6) has attributed the differences in growth rate of scions on different stocks to properties of the stem of the stock rather than to the root system. This is evidenced by certain double-worked apple trees, where the scion acquires the growth habit of the intermediate piece of grafted stem. Since it is

possible to double-graft etiolated peas (5), intermediate stem pieces of different varieties were grafted, and the effects on leaf, stipule, and stem growth of scion were measured.

Four experiments, totaling 826 double-grafted peas, were carried out to determine the interstock effect. Only 9 per cent of the total number of double grafts took, but the results obtained with these 76 successful grafts are sufficient to indicate that an intermediate stem piece about 50 mm. long does not affect scion development to any measurable extent. In one experiment, stock was Little Marvel and scion Alaska; in two others both stock and scion were Alaska; and in the last experiment Stratagem was used as stock and scion. There were no systematic differences in the development of the scion in these four combinations when different interstocks were grafted between them, so that the results are combined in table 3. To make all data comparable, the effect of Alaska as interstock has been taken as 100, so that the effects of Perfection, Stratagem, and Little Marvel are expressed in percentages of Alaska, a procedure previously followed (12). The data for comparison of these effects with those of the same varieties used as stock were obtained by combining data of this paper with those of the previous one.

Whereas Little Marvel—and especially Perfection—reduces stem growth when used as stock, they do not do so at all when used as interstock. When Alaska, Stratagem, Little Marvel, and Perfection are used as intermediate pieces, the differences in stem growth of scion are not great enough to indicate that any effect exists. The same can be said for leaf and stipule growth. But Stratagem and Little Marvel, when used as stock, definitely increase leaf growth.

If the intermediate stem piece were of any importance in determining the rate of development of the scion, it would make a difference whether a long scion were grafted on a short stock or a short scion on a long stock. Alaska peas were used as stock and Little Marvel as scion. One group had a 14-mm. stock and a 70-mm. scion; the other group had a 65-mm. stock and a 20-mm. scion. In both groups nine grafts took out of twenty; maximal growth rates were 14.4 ± 1.0 mm./day for long and 14.0 ± 0.6 mm./day for short scions. Leaf and stipule

TABLE 3

GROWTH OF PEA SEEDLING SCIONS EITHER GRAFTED ON VARIETIES MENTIONED OR ON SAME STOCK BUT WITH INTERMEDIATE STEM PIECE (DOUBLE-GRAFT). DATA FOR STOCK EFFECTS BASED ON ALL PEA-GRAFTING EXPERIMENTS TO DATE

STOCK OR INTERSTOCK	GROWTH IN LENGTH OF SCION EXPRESSED AS PERCENTAGE OF EFFECT OF ALASKA								SUCCESSFUL DOUBLE GRAFTS
	Stock (single grafts, data from this paper and 12)				Interstock (data from 826 double-grafted peas)				
	Stem	Leaf	Stipule	Petiole	Stem	Leaf	Stipule	Petiole	
Alaska	100	100	100	100	100 ± 3	100 ± 5	100	100	18
Stratagem	95	129	121	114	102 ± 6	105 ± 6	100	100	26
Little Marvel	84	122	103	105	108	108	.....	.....	7
Perfection	63	109	103	105	109 ± 6	100 ± 5	97	109	13

surfaces of second, third, and fourth leaves combined were 50 and 33 mm.² for long and 52 and 38 mm.² for short scions, showing that under these conditions also no effect of an intermediate piece was found on the scion.

If these experiments are considered together, they indicate that there is no systematic difference in the growth of the scion when different varieties are used as intermediate stem pieces. This means that varieties which as stock greatly influence leaf growth or growth in length of the scion do not do so by means of the character of their stem or by the type of graft union. Only their root system and cotyledons can be responsible for the development of the scion. This is the same conclusion as reached in the earlier papers, on different grounds, and of course holds only for these etiolated peas, where the growth rate of the scion is limited by the supply of growth factors from the seed and not by the rate of translocation through stem or graft union, as seems to be the case in double-worked apple trees.

Correlation between auxin content, growth rate, and nature of stock and scion

Two extensive grafting experiments, one comprising 560 grafts and the other 400, were carried out to determine the correlation of auxin content with growth rate. When the scions were growing rapidly, their 15-mm. long tips were cut off and placed in redistilled ether. At least five tips had to be extracted simultaneously to obtain a significant amount of auxin. The extract was taken up in 0.3, 0.5, or 1.0 cc. of 1.5 per cent agar and poured into two sets of twelve blocks, so that each extraction was assayed on twenty-four test plants.

In the first of these experiments, stocks of Alaska, Daisy, Little Marvel, Morse's 200, and Perfection were grafted with scions of either Alaska, Daisy, or Little Marvel. Forty grafts of each combination were made, and in the different groups 22–70 per cent of all grafts succeeded, with an overall efficiency of 36 per cent (table 4). The auxin content of the tips is given as indoleacetic-acid equivalents (10), which means that such concentration of the acid, if present in the tips, would have given the same curvature as that observed. At the time of extraction not all scions had the same growth rate, and this might affect the results. For this reason in most cases the tips were divided into two or three groups, with high, medium, and low growth rates. But no significant correlation between auxin content and growth rate was found. The auxin content is not related to the stock variety, to the growth rate, or to the scion variety. Since there are strict correlations between stock and scion variety on the one hand and growth rate on the other, it is evident that auxin is not limiting the growth rate in these scions. This confirms the earlier conclusion (12) that the growth rate of the scions is determined by a growth factor other than auxin, coming from the stock.

In table 4 the data for stem growth, leaf, stipule, petiole, and tendril development in general confirm those already presented (12). There is one general difference: the Alaska stocks give a relatively greater leaf and stipule growth compared with the other root stocks. This might be attributed to the fact that all the other stocks are from the same seeds as used in the former paper, whereas the Alaska stocks come from a new batch of seeds, of a harvest one or two years later.

Daisy as root stock produces in 95 per

TABLE 4

PEA SEEDLING SCIONS GRAFTED APRIL 19–21, 1938, AND MEASURED 2 WEEKS LATER. FORTY SEEDLINGS GRAFTED PER GROUP

Root stock	Scion	Percentage successful grafts	Maximal growth rate (mm./day)	Auxin content of stem tips*	Second leaf				Third leaf				Fourth leaf			
					Leaf surface (mm.²)	Stipule surface (mm.²)	Petiole length (mm.)	Tendril length (mm.)	Leaf surface (mm.²)	Stipule surface (mm.²)	Petiole length (mm.)	Tendril length (mm.)	Leaf surface (mm.²)	Stipule surface (mm.²)	Petiole length (mm.)	Tendril length (mm.)
Daisy	Alaska	25	38.7±5.3	36	46	33	6.2	4.8	52	37	13.7	8.0	.....	.....	.....	.....
Morse's 200	Alaska	22	25.4±2.0	44	22	22	4.3	3.2	27	24	7.7	7.0	.....	.....	.....	.....
Alaska	Alaska	25	37.7±1.8	32	30	28	4.7	3.8	36	26	12.3	7.9	.....	.....	.....	.....
Little Marvel	Alaska	25	35.7±3.7	45	50	35	6.2	4.7	50	36	8.8	7.6	.....	.....	.....	.....
Perfection	Alaska	22	17.6±0.6	34	21	19	4.0	3.4	16	16	3.5	4.2	.....	.....	.....	.....
Daisy	Daisy	35	10.4±0.7	36	72	42	5.1	3.2	103	83	8.8	5.5	100	63	9.2	11.2
Morse's 200	Daisy	60	11.0±0.6	5	50	35	5.0	2.6	77	53	7.5	5.4	61	56	10.0	9.5
Alaska	Daisy	70	10.8±0.4	24	44	34	4.4	3.3	61	39	6.0	5.6	66	43	8.4	10.4
Little Marvel	Daisy	32	8.1±0.5	17	69	46	5.4	3.8	61	40	5.8	5.1	74	33	7.6	10.3
Perfection	Daisy	32	6.2±0.6	28	29	25	3.7	2.7	37	20	4.0	4.8	.....	.....	.....	.....
Daisy	Little Marvel	35	9.9±0.8	35	.....	.....	.....	.....	69	64	8.8	8.9	121	75	11.8	14.6
Morse's 200	Little Marvel	50	10.0±0.6	.....	.....	.....	.....	.....	82	56	9.2	9.6	.....	.....	.....	.....
Alaska	Little Marvel	40	9.5±0.4	32	.....	.....	.....	.....	72	51	7.5	7.4	91	50	10.4	12.9
Little Marvel	Little Marvel	30	8.2±0.5	7	.....	.....	.....	.....	50	44	6.1	8.1	77	38	8.1	8.7

* In 10^{-4} gamma/kg. indoleacetic-acid equivalents.

cent of the cases larger leaves and stipules and longer petioles than when Alaska is used as root stock, although the stem growth rate of scions is the same for both root stocks. Little Marvel and Perfection both decrease growth in length, compared with Alaska stocks.

Since this experiment might be taken as an indication that upon storage the growth-factor content of pea seeds decreases, seed samples of the same lines but of different ages were used (obtained through the courtesy of the Ferry-Morse Seed Company). The seeds were superficially sterilized and after 6 hours' soaking in water were laid out on moist filter paper. Especially of the older seeds, a considerable percentage rotted before germination; in some instances no seedlings were obtained at all. After germination, the seeds were transferred to 20-cc. bottles with a small amount of water, and the growth rate was measured before grafting (table 5). The grafting was carried out in the regular manner, and maximal growth rate and leaf, stipule, petiole, and tendril size were determined in the scions of the successful grafts. In this experiment the auxin content is expressed in 10^{-4} gamma indoleacetic-acid equivalents per tip, the method of extraction and determination being the same as in the previous experiment (table 4).

The most important results indicated in table 5 are the following: (*a*) There is no consistent decrease in either growth rate or leaf development of scions grafted on older seeds, nor does the percentage of successful grafts fall off. In some cases less auxin is extracted from such scions, but in others not. (*b*) With aging, the percentage of rotting seeds (which are superficially sterilized) rapidly increases, but there is no indication of a decreasing growth-factor content of the seeds involved. The shoots which developed on the older seeds were perfectly normal, but owing to early infection most of them never grew at all. (*c*) There is no correlation between maximal growth rate of the scion and its auxin content.

Data on root formation are included in table 5. The successful scions were cut in sections 50–80 mm. long and placed overnight with their base in 200 mg./l. indoleacetic acid. For the next four days they stood in 2 per cent sucrose, and after that in 2 per cent sucrose with 0.01 mg./l. thiamin added. Alaska stems formed on an average more roots per cutting than did Daisy stems, but there is no obvious correlation between rooting and root stock. The data were also analyzed in a different way. Earlier experiments have shown (13) that in addition to a number of chemically identified factors, root formation in pea stems requires an unknown factor, rhizocaline, which is supplied from the cotyledons and which gradually disappears when the connection between stem and cotyledon is severed. Which relation exists between the taking of the graft and the rooting response of the scion? Very few stems rooted which had only recently started to grow and had not yet reached their maximal growth rate. The best rooting was obtained on the scions growing at their maximal rate. When the growth rate of scion was decreasing again, owing to either exhaustion of stock or breakdown of the graft union (evidenced by brown decoloration), rooting also became less abundant. This indicates that rhizocaline also moves across a graft union, perhaps slightly lagging behind caulocaline, and that a continuous supply from the stock is needed to keep up the ability of scions to root.

Nitrogen metabolism of graft partners

Determinations of the nitrogen fractions of scions were carried out by Dr.

TABLE 5

PEA SEEDLING SCIONS GRAFTED DECEMBER, 1938, AND MEASURED 2 WEEKS LATER. FORTY SEEDLINGS GRAFTED PER GROUP

Stock	Scion	Growth rate of stock before grafting	Stocks rotted before grafting (%)	No. of scions growing	Percentage successful grafts	Maximal growth rate of scion	Auxin content of scion per tip*	Root formation on scions	Second leaf surface (mm.²)	Second stipule surface (mm.²)	Second petiole length (mm.)	Second tendril length (mm.)	Third leaf surface (mm.²)	Third stipule surface (mm.²)	Third petiole length (mm.)	Third tendril length (mm.)
Alaska 1937.....	Daisy	18.4	2	25	66	15.4	7.5	1.7	57	32	5.3	3.3	63	41	6.0	4.8
Alaska 1936.....	Daisy	18.0	0	23	60	16.8	11.5	1.9	43	26	4.9	3.3	59	36	6.3	4.7
Alaska 1935.....	Daisy	19.0	3	15	39	17.1	8.3	0.9	35	27	4.5	3.3	44	30	6.3	5.0
Hundredfold 1937..	Daisy	2.1	49	3	23	13	.....	.....	90	69	7.7	4.7	135	101	11.7	9.0
Hundredfold 1936..	Daisy	3.3	37	3	21	11	.....	.....	115	80	8.2	7.2	173	48	11.0	10.0
Hundredfold 1935..	Daisy	2.8	50	4	25	.....	.....	.....	92	62	6.5	3.2	116	75	12.0	7.0
Stratagem 1938....	Daisy	9.8	17	15	42	15.9	32.1	0.6	70	40	6.0	3.6	85	54	8.4	6.3
Stratagem 1936....	Daisy	8.0	23	12	40	13.5	19.7	0.8	83	54	5.9	4.4	99	49	8.6	6.5
Perfection 1937.....	Daisy	10.2	15	9	25	10.1	.....	.....	54	30	4.5	3.3	57	21	5.2	5.4
Alaska 1937.....	Alaska	18.4	2	8	44	32.7	10.5	1.9	39	23	6.6	5.7	40	31	12.8	7.5
Alaska 1936.....	Alaska	18.0	0	7	41	41.0	4.5	10.5	42	31	5.6	4.5	35	25	10.1	6.7
Alaska 1935.....	Alaska	19.0	3	8	40	40.5	5.4	1.4	38	25	6.1	4.3	53	34	15.3	8.8
Stratagem 1938....	Alaska	9.8	17	4	23	34.7	29.0	10.0	50	28	6.4	4.7	.....	.....	.....	.....
Stratagem 1936....	Alaska	8.0	23	6	43	30.2	1.8	2.0	57	27	6.9	3.1	.....	.....	.....	.....
Perfection 1937.....	Alaska	10.2	15	10	67	30.0	10.2	2.3	50	22	5.2	2.1	33	18	7.9	5.7

* In 10^{-4} gamma/kg. indoleacetic-acid equivalents.

H. BORSOOK. The results in table 6 show that increase in total nitrogen is completely correlated with growth of the scion. Before the graft had taken, no change in nitrogen content of the scion occurred. But later, depending on how much growth had taken place, more or less nitrogen reached the scion. There was a corresponding decrease in the nitrogen content of the seed, but this decrease occurred also before the graft succeeded.

TABLE 6

NITROGEN FRACTIONS (AS DETERMINED BY DR. BORSOOK) OF SCIONS AND COTYLEDONS OF GRAFTED PEAS. TOTAL NITROGEN REFERS TO MG. OF NITROGEN PER TEN SCIONS OR TEN PAIRS OF COTYLEDONS

Perfection tips on	Total growth (mm.)	Growth rate before analysis (mm./day)			Maximal growth rate (mm./day)	Time between grafting and analysis (days)	No. of tips analyzed	Scions				Cotyledons of stock			
		Three days	Two days	One day				Total N	Percentage total N			Total N	Percentage total N		
									Protein	Free amino	Amide		Protein	Free amino	Amide
(Before grafting)	.....	.....	.....	.....	.....	0	10	13.5	41	26	13	47.8	53	22	3
Perfection	4.3	0.2	1.0	2.8	3.0	11	10	13.4	22	30	16	38.6	42	28	6
Perfection	41.5	7.0	6.9	2.1	12.3	13	10	17.2	18	29	20	27.3	28	34	11
Perfection	46.5	8.7	7.8	9.3	12.0	13	10	25.7	20	32	19	21.9	24	37	11
Daisy	1.2	0.6	0.0	0.3	0.8	10	10	12.9	17	31	21	23.2	34	32	9
Daisy	45.4	6.4	6.4	7.0	12.5	18	5	23.2	19	29	20	.....	.....	.....	.....
Daisy	85.4	7.2	7.2	8.0	16.4	18	5	36.9	19	31	20	.....	.....	.....	.....

This is comprehensible, since the roots continue to grow on such stocks. The last recorded value of nitrogen in cotyledons is not comparable with the others, since it refers to seeds of Daisy instead of Perfection. The protein, amino and amide fractions of the total nitrogen are rather constant in the scions, after an initial drop in protein, and do not vary with the root stock, although the stem and leaf growth rates are very different.

ANALYSIS OF GENETIC CHARACTERS BY GRAFTING TECHNIQUE

As mentioned before (12), it would be interesting to use as root stock peas with genetically-known aberrant leaf or growth characters. In this way it would be possible to determine whether a specific gene affects the growth-factor supply or the reactivity of the tissues.

The first such case was investigated by DE HAAN and GORTER (4). They found that the double recessive Slender character (la, lb according to DE HAAN, 3) owes its excessive length, at least partly, to a decreased auxin destruction, when compared with the dominant allelomorph. WENT (12) showed that in addition Slender seeds had a greatly reduced supply of leaf and stipule growth factors. A new experiment with the last available seed supply was carried out, with substantially the same results as previously reported (table 7). This table indicates that the non-Slender plants of the same family as Slender (Tall-and-Short), and probably the Slender plants as well, are a much better source for caulocaline than is Alaska. Whereas Tall-and-Short serves the scions with as much leaf and stipule growth factors as Alaska, the Slender stocks are deficient in these fac-

tors. The stem-growth response of Slender to caulocaline is the same as that of Alaska, but the reactivity of the stipule is definitely less. In Tall-and-Short the reactivity to caulocaline is decreased, showing that the Slender character is pleiotropic; it decreases auxin destruction, decreases leaf and stipule growth factors stored in seeds, and increases reactivity of the stem to caulocaline. Furthermore, according to DE HAAN (3) it decreases chlorophyll content, increases peduncle growth, and causes flower abnormalities.

TABLE 7

STOCK AND SCION EFFECTS OF ALASKA PEAS GRAFTED ON OR WITH PEAS SHOWING A 3:1 SEGREGATION IN TALL-AND-SHORT AND SLENDER

Stock	Data	Scion: Alaska	Scion: Tall-and-Short	Scion: Slender
Alaska	Successful grafts	18	13	5
	Growth rate*	25.9±1.7	15.2±0.8	25.2±5.7
	Leaf length†	7.0±0.3	8.5±0.6	8.4±0.4
	Stipule length	7.3±0.2	7.6±0.2	5.8±0.5
	Petiole length	5.1	5.7	8.3
Tall-and-Short	Successful grafts	15	5	.....
	Growth rate	37.5±1.5	19.8±2.2	.....
	Leaf length	6.9±0.25	8.1±0.9	.....
	Stipule length	7.2±0.2	8.3±0.4	.....
	Petiole length	6.3	6.4	.....
Slender	Successful grafts	5	.....	.....
	Growth rate	36.0±4.2	.....	.....
	Leaf length	6.0±0.5	.....	.....
	Stipule length	6.2±0.4	.....	.....
	Petiole length	5.1	.....	.....

* Maximal rates reached by scions in mm./day. † Length measurements in mm.

Among other genetically-analyzed growth characters of the pea can be mentioned the Tall-Dwarf character, which expresses itself in the number and length of internodes, but Tall is not nearly so extreme as the Slender type of growth. This has not been investigated, neither has the Fasciated Stem.

A few cases of Stipuleless have been described. Two of them were investigated. One was Acacia-leaf, in which the leaves are multijugate by metamorphosis of tendril branches into leaflets. It also was Stipuleless. A number of these plants, received through the courtesy of Miss PELLEW and coming from different strains, were germinated and grafted with Alaska. Leaf growth was little affected by Stipuleless stock, and the stipule growth of Alaska scions was equal with both stocks. Stipuleless did not develop larger stipules on Alaska stock. This shows that Stipuleless is due to a lack of growth response of the primordium to stipule growth factors, which are supplied in sufficient amount by the cotyledons. In conjunction with decreased stem-growth rate, the petiole development was much smaller when Acacia-leaf was used as stock, and the same is true for tendril development. This may be attributable to the fact that in this case Stipuleless was of the Acacia-leaf type, which has small tendrils. The character of Acacia-leaf is not transmitted across a graft union, since the number of leaflets of Alaska was not increased by this root stock.

In table 8 two experiments are summarized in which two genetic characters are compared in their effects on leaf and stipule growth. These races of Stipuleless were obtained through the courtesy of Dr. BARBER. There is a slight but significant decrease in stipule growth when Stipuleless stocks are used (table 8). Growth of leaves and stems, however, is not significantly influenced by this stock.

TABLE 8

GROWTH RATES AND MEAN LEAF, STIPULE, AND PETIOLE SIZES OF FIRST TO THIRD LEAVES OF RECIPROCAL GRAFTS OF MUTANTS AND RACES OF PEA VARIETIES. MEAN OF TWO EXPERIMENTS

Stock	Scion	No. of successful grafts	Maximal growth rate		Mean length of first to third nodes of scion			
			Mm./day	Reached after (days)	Leaf	Stipule	Petiole	Tendril
Alaska	Alaska	38	36	8.3	7.6	7.7±0.2	6.1	4.2
Duke of Albany (type)	Alaska	9	34	7.3	7.5	7.1	5.6	4.4
Duke of Albany Stipuleless	Alaska	32	35	7.3	6.9	6.0±0.2	5.1	3.2
Duke of Albany Rogue	Alaska	25	38	8.0	7.1	6.7±0.4	5.6	4.7
Alaska	Duke of Albany Stipuleless	21	39	7.2	7.7	2.3	6.8	6.3
Alaska	Duke of Albany Rogue	20	39	7.0	5.5	5.5	6.1	5.1
Duke of Albany Stipuleless	Duke of Albany Stipuleless	15	41	.....	7.0	2.4	8.0	.....
Duke of Albany Rogue	Duke of Albany Rogue	7	25	.....	5.2	6.1	6.0	.....

Rogue is a dominant character, which causes reduction of leaf and stipule growth. This character is due exclusively to reduced reactivity of the Rogue leaflets and stipules to leaf and stipule growth factors (table 8), and the latter are present in the cotyledons in the same amounts as in the parent variety from which the Rogue originated.

The results obtained in these tests with strains differing in only one gene are in marked contrast with a comparison between pea varieties as summarized in table 13 of the first paper of this series (12). In the latter case it was concluded that the varieties differed both in the response of their different organs to growth factors as well as in the growth-factor content of their seeds. Both mechanisms contributed about equally to the size and shape of the seedling.

The case of Slender is complex, a situation in which reactivity of the leaves and stipules is decreased and that of the stem increased, together with a decrease of the growth-factor content of seeds and an increase in the caulocaline production of the roots. The Slender habit is certainly more than the decreased auxin destruction described by DE HAAN and GORTER (4). It is evident that all these pleiotropic effects must be based on one much more fundamental effect of the Slender gene.

In the other three genes investigated (Stipuleless, Acacia-leaf, and Rogue) a simple relationship between gene and effect existed. In none of them was there a considerable change in the growth-factor content of the seeds. Practically the whole effect was due to a change in responsiveness of the tissues under control

of the gene. In *Zea mays* the growth substance analysis of a number of dwarf races (8, 9) gave a similar result; the dwarf genes did not affect the auxin content as such, or the auxin synthesis, but the dwarf habit was due mainly to excessive auxin destruction. Between various corn varieties, however, quantitative differences in auxin content exist.

These data indicate that differences in growth habit, which are due to known genes, are not primarily caused by differences in growth-factor synthesis or content. It is evident that growth-factor differences could account for major differences in habit, but also they would be controlled to a considerable extent by external conditions. There are two possible explanations of the one-sided nature of gene action in affecting the growth habit in peas:

1. Since growth-factor differences are so much influenced by growing conditions, clear-cut genetic data are hard to obtain, so that genes controlling the quantity of growth factors have not been picked up by geneticists.

2. Genes affecting size and form in peas are influencing the response of the individual cells to growth factors, or in corn they cause destruction of auxin. However, quantitative differences in growth-factor content do not seem to be controlled by known gene differences. It must therefore be assumed that the varietal differences in growth-factor content are due to more complicated genetic relations. In the few cases that quantitative characters have been genetically analyzed in plants, complex situations are encountered, calling for a whole array of multiple allelomorphs with modifying factors (7).

As soon as more genetic characters affecting size of stem, leaves, stipules, and tendrils of peas are known, this problem can be investigated in greater detail.

Growth-factor content of peas soaked in water

When peas are soaked in water for prolonged periods, leaf-growth factors leach out of the cotyledons (2). Such soaked peas germinate poorly or not at all. This compares with the behavior of peas sown in the field, which germinate very poorly if rain drenches the soil soon after sowing. The question was whether this poor germination could be accounted for by the depletion of growth factors in the cotyledons, or whether any tissues were injured so that no further growth could occur.

Superficially disinfected peas were submerged in an excess of water (about 100 seeds per 150 cc. of water) for various periods, partly without and partly with aeration. In the latter case a strong current of air (± 100 cc. per minute) was bubbled aseptically through the container. After the period of soaking (1, 2, 3, 4, 6, or 8 days), the peas were laid out in the usual way on filter paper and after germination transferred to 20-cc. wide-mouthed bottles. When the plants had reached the proper length, all graft combinations between controls (soaked 4 hours only) and treated peas were made. In the first experiment, 201 out of 263 grafts succeeded; in the second, 190 grafts took. Since both experiments gave essentially the same results, only one will be described, based on the data of table 9. The upper three lines define the conditions to which the peas were subjected previous to germination. In groups 4 and 5 considerable growth of the seedlings occurred even while submerged, but the developing shoots were thinner and had very small leaves, stipules, petioles, and tendrils. When soaked for more than 50 hours in unaerated water, no further growth occurred after removal of the seeds from the solution (groups 8 and 9). From

line 4 it follows that considerable substance diffused from the peas, but part of this was reabsorbed by the developing seedlings in groups 4 and 5. Part of the material diffusing out was leaf-growth factor, as the determinations of line 5 show (carried out by Dr. D. BONNER with the standard leaf-growth test method, 1). The longer the peas were soaked the more leaf-growth factor diffused out, especially when the water was not aerated. Lines 6–12 indicate the condition of the soaked peas after they had germinated and grown. Soaking greatly retarded early growth of the seedlings, so that it took 6–92 hours longer than the controls to reach a length of 30 mm. But once they started to grow

TABLE 9

GROWTH AND DEVELOPMENT OF PEAS SOAKED IN AERATED OR NONAERATED WATER, COMPARED WITH CONTROLS SOAKED 4-HOUR PERIOD ONLY (GROUP 1). UPPER 13 LINES REFER TO BEHAVIOR OF PEAS PRIOR TO GRAFTING. FROM 8–18 GRAFTS OF EACH COMBINATION WERE SUCCESSFUL. LOWER 10 LINES GIVE MEASUREMENTS OF SCIONS OF SUCCESSFUL GRAFTS. LINES 14, 17, 19, AND 22 REFER TO GRAFTS IN WHICH STOCK BELONGED TO SOAKED GROUPS AND SCION TO CONTROLS, WHEREAS IN LINES 15, 18, 21, AND 23 THE STOCKS WERE CONTROLS (FROM GROUP 1) AND THE SCIONS SOAKED

LINE	DATA			GROUP								
				1	2	3	4	5	6	7	8	9
1	Hours soaked			4	24	48	96	144	24	48	96	144
2	Aerated or not			.....	Air	Air	Air	Air	No air	No air	No air	No air
3	Subsequent germination			++	++	++	+	+	+	+	−	−
4	Diffusate in soaking water (mg.)			.....	45	62	48	30	65	83	98	240
5	Amount of leaf-growth factor in diffusate (percentage growth)			0	1.3	1.3	2.3	2.3	2.7	3.3	4.3	5.7
6	Hours after beginning of soaking for seedlings to reach 30 mm. length			131	137	159	189	223	168	205	.....	.....
7	Lag of treated seedlings behind controls (hours)			.....	6	28	58	92	37	74	.....	.....
8	Growth rate of seedlings (20–50 mm.)			36	30	36	36	.....	33	40	.....	.....
9	Development of organs at fourth node	Leaf		8.1	8.1	6.7	5.4	4.5	6.8	6.4	.....	.....
10		Stipule		7.3	6.9	5.4	4.4	3.8	5.7	5.2	.....	.....
11	Length in mm.	Petiole		7.8	7.2	4.6	3.4	2.8	4.3	4.2	.....	.....
12		Tendril		4.7	3.0	2.6	1.5	1.3	2.7	2.1	.....	.....
13	Diameter of stem of soaked seedling			2.28	2.28	2.24	2.04	2.16	2.20	2.18	.....	.....
14	Effects of stock on scion development: Maximum growth rate (mm./day) when soaked seedling is	Stock		40	39	35	40	40	37	39	.....	.....
15		Scion		40	40	39	40	40	36	42	.....	.....
16		Both stock and scion		40	38	38	38	.....	39	.....	.....	.....
17	Leaf (mean of third and fourth leaves) length (mm.) soaked seedling being	Stock		7.0	7.0	7.1	6.5	6.7	6.3	5.6	.....	.....
18		Scion		7.0	7.3	7.0	6.3	6.4	6.9	7.1	.....	.....
19		Both stock and scion		7.0	.....	7.3	6.2	.....	6.0	.....	.....	.....
20	Mean length of third and fourth stipules when soaked seedling is	Stock		7.1	7.1	7.7	7.3	6.9	7.4	6.5	.....	.....
21		Scion		7.1	7.0	7.3	7.3	7.1	6.9	7.4	.....	.....
22	Mean length of third and fourth petioles when soaked seedling is	Stock		10.7	9.0	7.9	7.3	6.4	7.8	4.8	.....	.....
23		Scion		10.7	8.3	7.3	6.1	6.7	8.7	8.3	.....	.....

their growth rate was normal and approximately the same, irrespective of soaking. Only those seedlings which developed normal roots started to grow, whereas those with roots permanently injured by the soaking hardly grew at all. This supports the view earlier expressed (13) that only in actively growing pea roots is an essential growth factor for stems (caulocaline) formed. As can be seen from table 9, there were great differences in the appearance of the seedlings, even though they grew at the same rate: the longer they were soaked the less leaf, stipule, petiole, and tendril development occurred, and the thinner were the stems.

After grafting, the growth rates of the scions were the same, whether soaked peas were used as stock, as scion, or as both stock and scion (table 9). The remarkable uniformity of the maximal growth rates indicates how well the grafting in this experiment succeeded (201 out of 263 grafts took—a mean of 12 per group). To judge the effect of the grafting on leaf, stipule, and petiole development, the mean length of the third and fourth leaves on each graft was taken. From the third leaf on, the effect of the soaking itself has mostly worn off, so that the stock effects become apparent. It will be seen that especially the nonaerated peas when used as stock decrease leaf and petiole growth of the nonsoaked scion. If the leaf length of the fourth leaves of nonsoaked scions grafted on groups 4, 5, 6, and 7 (6.66 ± 0.20) is compared with the length of the fourth leaves of scions grafted on controls (group 1: 7.50 ± 0.20), then the difference between these groups is highly significant ($\Delta = 0.84 \pm 0.27$), indicating that the content of leaf-growth factor has significantly decreased during soaking, which was to be expected since these factors could be collected from the water in which the material was soaked. Comparison of lines 5 and 17 shows that there is a rough proportionality between the amount of leaf-growth factors in the water and the amount which disappeared from the cotyledons. The decreased leaf development in groups 4 and 5, line 18, was due mainly to incomplete recovery of the third leaf from soaking, since the fourth leaves were already of almost the same size as those in the control grafts.

Conclusions reached from this experiment, confirmed by those of another, are that soaking peas for prolonged periods decreases subsequent germination, especially when the water is not aerated. The effects of this soaking are either (*a*) transitory or (*b*) permanent. Whenever the roots have been injured beyond recovery, no further stem growth is possible. But when the roots recover, growth of the stem, both prior to and after grafting, becomes normal and maximal. The inhibition of growth in length during the soaking period is therefore transitory and can be traced back to temporary injury to the roots resulting in a temporary decrease in caulocaline production. The decreased development of leaves, stipules, petioles, and tendrils in the soaked material is not due to injury to the cells, for when grafted on the control seedlings their growth becomes normal again. Consequently, the smaller size of these organs has to be attributed to a decreased supply of growth factors, which leach out into the water; the leaching is much greater for the shoots than for the cotyledons.

This soaking produces the same condition of reduced organ size in peas as some of the genetic characters which decrease leaf and stipule growth. But whereas the growth genes cause a de-

creased reactivity of the cells, without affecting the leaf-growth factors, soaking mainly decreases growth factors without affecting the reactivity of the cells.

Summary

1. The percentage of successful grafts of pea seedlings grown in darkness at 24° C. and 80 per cent humidity drops rapidly when the plants are more than 8 days old, both when used as stock or as scion. No considerable improvement in percentage of take or of subsequent maximal growth rate is observed when the cut surfaces of the graft partners are treated with plant hormones. When stem pieces of varieties which as stock produce considerable differences in scion growth are grafted as intermediate stems between pieces of one test variety, they do not influence the scion. The effect of stock on scion is exclusively due to cotyledons and root system and not to transport properties of the stem or to the graft union, once this is established.

2. There is no correlation between auxin content of the tip on the one hand and stem growth rate and stock variety on the other hand, indicating that the growth rate in these grafts is not limited by auxin but by another factor coming from the stock. This is caulocaline, as concluded in the first paper of this series.

3. The decrease in viability of pea seeds upon storage is not due to a gradual decrease in growth factors present in the cotyledons. The only observable effect of age is decreased resistance to premature rotting of the seeds.

4. The protein content of the scion follows very closely its growth rate.

5. The genetic characters Stipuleless, Acacia-leaf, and Rogue are not accompanied by appreciable changes in growth-factor content of the stocks; they are due almost entirely to changes in reactivity of the tissues. On the other hand, decreased development of leaves, stipules, petioles, and tendrils due to soaking of the peas in water for 1–6 days is caused by decrease in growth-factor content of the cotyledons and not by changes in reactivity of the tissues to growth substances.

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LITERATURE CITED

1. Bonner, D. M., and Haagen-Smit, A. J., Leaf growth factors. II. The activity of pure substances in leaf growth. Proc. Nat. Acad. Sci. **25**:184–188. 1939.
2. Bonner, D. M., Haagen-Smit, A, J., and Went, F. W., Leaf growth hormones. I. A bioassay and source for leaf growth factors. Bot. Gaz. **101**:128–144. 1939.
3. De Haan, H., Length factors in *Pisum*. Genetica **9**:481–498. 1927.
4. De Haan, I., and Gorter, C. J., On the differences in longitudinal growth of some varieties of *Pisum sativum*. Rec. Trav. Bot. Néerl. **33**:434–446. 1936.
5. Hayward, H. E., and Went, F. W., Transplantation experiments with peas. II. Bot. Gaz. **100**:788–801. 1939.
6. Roberts, R. H., Some stock and scion observations on apple trees. Wisconsin Agr. Exp. Sta. Res. Bull. 94. 1929.
7. Sirks, M. J., Growth and inheritance of leaf-dimensions in the broad-bean (*Vicia faba* L.). Proc. Kon. Akad. Wetensch. Amsterdam **32**: 1066–1084. 1929.
8. van Overbeek, J., The growth hormone and the dwarf type of growth in corn. Proc. Nat. Acad. Sci. **21**:292–299. 1935.
9. ———, Auxin production in seedlings of dwarf maize. Plant Physiol. **13**:587–598. 1938.
10. ———, A simplified method for auxin extraction. Proc. Nat. Acad. Sci. **24**:42–46. 1938.
11. von Wettstein, F., and Pirschle, K., Über die Wirkung heteroplastischer Pfropfungen und die Übertragung eines Gen-bedingten Stoffes durch Pfropfung bei *Petunia*. Biol. Zentr. **58**:123–142. 1938.
12. Went, F. W., Transplantation experiments with peas. Amer. Jour. Bot. **25**:44–55. 1938.
13. ———, Specific factors other than auxin affecting growth and root formation. Plant Physiol. **13**:55–80. 1938.
14. ———, Effects of light on stem and leaf growth. Amer. Jour. Bot. **28**:83–95. 1941.

EFFECTS OF APPLICATION OF THIAMINE TO COSMOS[1]

JAMES BONNER

Introduction

Earlier experiments (2, 3) were thought to indicate that the application of thiamine to selected species of plants might result in increased growth under the environmental conditions used. In general, other investigators have not found such effect (4, 1, 5), although occasionally positive responses have been recorded (6, 7). The present investigation has been a study of some of the factors influencing the growth of *Cosmos sulphureus*, and the relation of these factors, if any, to the apparent growth response of *Cosmos* to added thiamine. In all, eighty-five experiments with the application of thiamine were conducted. In fifty of these experiments, growth responses which appeared to be significant were obtained. In the remaining thirty-five experiments no significant responses appeared. Of the several factors whose influence on the growth of *Cosmos* was investigated, certain ones appeared to bear little relation to any apparent response to thiamine. This was true of the pH of the nutrient medium over a considerable range, varying degrees of nitrogen deficiency, and light intensity within wide limits. The temperature relations of the conditions under which *Cosmos* was maintained, however, appeared to be of great importance in the growth of this plant.

Material and methods

All plants grown in sand culture were contained in 2-gallon glazed crocks provided with drainage. In most of the experiments a local washed river sand, having particles between 10 and 20 mesh, was used. Growth in this medium appeared to be as luxuriant as in 4-mesh gravel or in 4-mesh quartz gravel. Twenty seeds of *Cosmos*, var. Klondyke orange flare, were planted per crock. When the first foliage leaves were well developed (in general, 7–10 days after planting), the experiment proper was commenced. Thirty days later the plants were harvested, the tops separately from the roots, dried rapidly in a current of dry air at 60° C., and weighed.

Hoagland's nutrient solution containing the micro-nutrients was applied at the rate of about 500 cc. per crock per day or per 2 days. Experiments with varying frequency of nutrient application were conducted periodically throughout the investigation, and in no experiment—with the possible exception of C75 (table 4)—was growth limited by the amount of available nutrient. Additional water was supplied as needed, and the crocks were periodically flushed with water.

No consistent difference between growth of plants supplied with distilled water and that of plants supplied with tap water was found, but in most of the experiments distilled water was used. Thiamine was applied in the nutrient solution at the rate of 0.01 mg. per liter.

Two greenhouses were employed. In the first the temperature was controlled only in so far as a night minimum of 55° F. was maintained. In the second, an air-conditioned greenhouse, constant temperatures and humidities could be maintained. The experiments in the air-conditioned greenhouse were carried out at a relative humidity of 70 per cent, regardless of temperature.

[1] Report of work done with the assistance of the Work Projects Administration, O.P. no. 165-1-07-172. This work was made possible in part by the support of Merck and Company.

A randomized block design was employed with all experiments. At the time of harvesting, the ten plants of each crock were divided into two lots of five plants each. Treatment of the data from typical experiments by the analysis of variance showed that variation between the two lots from the same crock was not significantly different from that between two lots from different crocks. The unit of five plants is therefore used as the basis for the computation of the standard errors in the tables. Any comparison, unless otherwise stated, is attended by fourteen or more degrees of freedom. The word significant is used only in its statistical sense.

TABLE 1

DEVELOPMENT OF COSMOS PLANTS WITH AND WITHOUT ADDITION OF THIAMINE TO NUTRIENT SOLUTION

EXPERIMENT NO.	TOTAL NO. OF PLANTS	DRY WEIGHT OF TOPS (GM. PER FIVE PLANTS)			DRY WEIGHT OF ROOTS (MG. PER FIVE PLANTS)		
		Control	Thiamine	Difference	Control	Thiamine	Difference
C48....	160	0.350±0.0138	0.485±0.0295	0.135±0.0326*	35.0±2.60	38.8±2.92	3.8±3.90
C49....	160	0.505±0.0214	0.655±0.0294	0.150±0.0364*	47.2±4.4	60.0±5.95	12.8±7.40
C50....	160	0.419±0.014	0.592±0.039	0.173±0.0413*	45.0±4.0	72.5±6.35	27.5±7.50*

* Difference significant at 1 % level.

Experimental results

REPETITION OF EARLIER EXPERIMENTS.—In certain experiments an attempt was made to repeat as closely as possible those reported earlier (3). *Cosmos* plants were grown in sand, ten plants per crock, in 2-gallon crocks in the greenhouse, and harvested after 30 days. Table 1 gives the data from three such experiments. Significant increases in dry weight of tops appear to have been obtained as a result of treatment, amounting to 39, 30, and 41 per cent, respectively, in the three experiments. Effect of the treatment on dry weight of roots was variable and not significant in two of the three experiments. This experiment was repeated fifteen times and yielded a mean increase of dry weight of tops as a result of treatment of 27±4.4 per cent, a highly significant result. Although the effect of the treatment varied from experiment to experiment, it would appear that under the conditions used a small but significant increase in dry weight of tops resulted from thiamine application in individual experiments and in the whole series.

EFFECT OF TEMPERATURE.—It has been pointed out by HAMNER (4) that the *Cosmos* plants grown in the earlier experiments of BONNER and GREENE (3) exhibited less luxuriant growth than those grown by himself. The same criticism applies to the plants of table 1. This low rate of growth was not due to a limitation of the available nutrient, but was related to the relatively low temperatures which were, for other reasons, maintained in the greenhouse. Thus, the nightly minimum through the winter months, during the earlier experiments, was approximately 55° and the daily maximum approximately 75° F. In preliminary experiments it was found that when *Cosmos* plants, under otherwise similar conditions, were allowed to develop either at the aforementioned temperature conditions or at a constant temperature (80° F.), growth was consider-

ably greater at the higher temperature. Experiments at varying temperatures were therefore undertaken. Two units of the air-conditioned greenhouse were set for constant temperatures of 80° and 68° F., respectively. In addition, plants were transferred between the two units, so that an 8-hour period per day at one temperature was combined with a 16-hour period per day at the other temperature. In all, ten experiments, each including treatment with two or more different temperature combinations, were carried out during two successive winters. Data from two such experiments are summarized in table 2. Plants maintained continuously at 68° appeared to show a small response to thiamine treatment. Plants maintained at 80° or which received a temperature of 80° during the night and 68° during the day showed no significant response to the treatment with thiamine.

In the two experiments of table 2, growth does not appear to be greatly affected by the several temperature treatments. When a day temperature of 80° was combined with a night temperature of 68°, however, growth was measurably improved over that obtained at a continuous temperature of 68° (table 3).

TABLE 2

DEVELOPMENT OF COSMOS PLANTS AT 80° AND 68° F. WITH AND WITHOUT ADDITION OF THIAMINE TO NUTRIENT SOLUTION

Experiment no.	Temperature		Total no. of plants	Dry weight of tops (gm. per five plants)			Dry weight of roots (gm. per five plants)		
	Day	Night		Control	B_1	Difference	Control	B_1	Difference
C60.....	68°	68°	80	0.95±0.065	1.30±0.047	0.35±0.080*	0.22±0.018	0.34±0.012	0.12 ±0.022*
C66.....	68°	68°	80	0.80±0.063	1.18±0.080	0.38±0.102*	0.16±0.022	0.20±0.019	0.040±0.029
C60.....	68°	80°	100	0.91±0.064	1.04±0.042	0.13±0.077	0.24±0.020	0.28±0.017	0.040±0.026
C66.....	80°	80°	80	1.08±0.12	1.18±0.15	0.10±0.19	0.15±0.018	0.16±0.023	0.010±0.029

* Difference significant at 1% level.

TABLE 3

DEVELOPMENT OF COSMOS PLANTS AT 80° AND 68° F. WITH AND WITHOUT ADDITION OF THIAMINE TO NUTRIENT SOLUTION

Experiment no.	Temperature		Total no. of plants	Dry weight of tops (gm. per five plants)			Dry weight of roots (gm. per five plants)		
	Day	Night		Control	B_1	Difference	Control	B_1	Difference
C67.....	68°	68°	80	2.30±0.12	3.21±0.14	0.91±0.18*	0.33±0.054	0.46±0.038	0.13 ±0.066
C71.....	68°	68°	40	2.65±0.13	3.18±0.15	0.53±0.20†	0.39±0.069	0.35±0.076	0.040±0.103
C81.....	68°	68°	300	2.24±0.070	2.70±0.087	0.46±0.11*	0.28±0.011	0.34±0.017	0.06 ±0.020*
C84.....	68°	68°	120	2.72±0.11	3.55±0.12	0.83±0.16*	0.39±0.022	0.40±0.020	0.010±0.030
C67.....	80°	68°	80	4.95±0.24	4.70±0.20	0.25±0.31	0.94±0.13	1.04±0.12	0.10±0.18
C81.....	80°	68°	120	5.73±0.30	6.30±0.29	0.57±0.42	0.76±0.065	0.74±0.051	0.02±0.082

* Difference significant at 1% level. † Difference significant at 5% level.

In the four experiments of table 3, growth at a continuous temperature of 68° was greater than that in the comparable experiments of table 2, owing to factors as yet unknown. With a day temperature of 80° and a night temperature of 68°, growth was still greater. As in the experiments of table 2, a significant or highly significant response to thiamine treatment (with respect to dry weight of tops produced) was obtained at the continuous temperature of 68°. Plants which received a day temperature of 80° and a night temperature of 68° exhibited no response to the treatment.

Dry weight of roots was affected variously, a significant result being obtained in only one case.

These experiments indicate that under the prevailing conditions the temperature relations may affect not only the growth of *Cosmos* but also its apparent response to thiamine treatment. Those temperature relations favoring more rapid growth do not appear to be conducive to any apparent response to thiamine (table 3).

LUXURIANT GROWTH.—Some effort was made to discover conditions favoring a growth of *Cosmos* still more luxuriant than that recorded in table 3. The number of plants per 2-gallon crock was reduced from ten to four, although this alteration resulted in but slight increase in growth. The most luxuriant growth as yet obtained was in midsummer and with the use of the system described by WENT (8), in which the prevailing temperature is adjusted to the prevailing light intensity. The maximum temperature in the greenhouse under full sunlight was approximately 90°, while the nightly minimum was 65°. The results of this experiment are given in table 4 (experiment C75). At the expiration of 30 days the plants were more than 3 feet tall, about ½ inch in diameter, and the dry weight of the tops was several times greater than that of the largest plants of table 3. No apparent response to thiamine was evident. Repetition of the experiment failed to duplicate such growth, and in one case (table 4, experiment C79)—carried out in the fall—a significant response to thiamine was apparent. Relatively rapid growth was also obtained in unaerated solution culture (table 4), in which case, in confirmation of the findings of ARNON (1), no response to thiamine was apparent.

TABLE 4

DEVELOPMENT OF COSMOS PLANTS UNDER VARIOUS CONDITIONS AND WITH OR WITHOUT ADDITION OF THIAMINE TO NUTRIENT SOLUTION

EXPERIMENT NO.	ENVIRONMENT	TOTAL NO. OF PLANTS	DRY WEIGHT OF TOPS (GM. PER FIVE PLANTS)			DRY WEIGHT OF ROOTS (GM. PER FIVE PLANTS)		
			Control	B_1	Difference	Control	B_1	Difference
C75.....	Balanced light temperature system (July)	120	21.1 ±0.78	21.8 ±0.75	0.7 ±1.08	3.38±0.20	3.50±0.22	0.12±0.30
C79.....	Balanced light temperature system (September)	96	7.10±0.26	8.75 ±0.35	1.65±0.43*	1.12±0.10	1.40±0.16	0.28±0.19
C78.....	Solution culture	36	7.88±0.415	7.72±0.285	0.16±0.503	2.21±0.157	2.14±0.169	0.07±0.231

* Difference significant at 1% level.

Discussion

Significant increases in growth of tops of *Cosmos* plants were obtained in certain experiments apparently as a result of the application of thiamine. Response to added thiamine, however, depended on the conditions under which the plants were grown. Of the environmental factors affecting the response, temperature would seem to be of greatest importance, so far as the present experiments are concerned. On the whole, temperature relations conducive to luxuriant growth would appear to be unfavorable for demonstration of any apparent response to added thiamine.

Even in experiments where apparent growth responses to added thiamine were found, these responses were smaller

than those brought about by suitable changes in temperature. Thus, at a continuous temperature of 68° F. the addition of thiamine in six experiments resulted in an apparent increase of 33 per cent in dry weight of tops, whereas subjecting similar plants to 8 hours per day at 80° resulted in an increase of more than 100 per cent.

The present discussion has centered on the growth responses of the tops of the plants, since responses in dry weight of roots were variable and in general insignificant (tables 1–4). No explanation of this fact can be given at present.

It is recognized that these experiments should be expanded to cover further temperatures and temperature combinations, as well as further variation of other environmental factors in combination with temperature. It is also recognized that other factors, as yet unknown, may exert important influences in determining the outcome of such experiments. It is possible, however, that the data presented here may suggest a partial explanation of the discrepant results obtained from thiamine applications to plants by other investigators.

The results of the present study are not held to bear on the question of the lack of practical value of promiscuous thiamine application to plants. Considerable published and unpublished evidence has established that such applications are without general merit.

Summary

1. Experiments on the application of thiamine to *Cosmos* plants were repeated under conditions similar to those obtaining in previous experiments (3). Addition of thiamine to the nutrient solution appeared to elicit small growth responses, as judged from dry weight of tops produced.

2. The relatively slow growth rate obtained in the earlier experiments (3) was found to be owing to the relatively low temperatures under which the plants were grown.

3. Thiamine was applied to plants grown at controlled temperatures of 80° and 68° F. Positive growth responses to added thiamine appeared to be obtained at a constant temperature of 68° but not of 80° F., nor at a day temperature of 80° combined with a night temperature of 68° F.

4. Temperature conditions favoring luxuriant growth appeared unfavorable for demonstration of any apparent growth-promoting effect of thiamine.

5. Although temperature conditions, in so far as they were studied, appeared to affect the apparent response to thiamine, it is recognized that other factors may have similar and possibly more striking effects.

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LITERATURE CITED

1. ARNON, D., Vitamin B_1 in relation to the growth of green plants. Science **92**:264–266. 1940.
2. BONNER, J., and GREENE, J., Experiments on the relation of vitamin B_1 to the growth of green plants. BOT. GAZ. **100**:226–237. 1938.
3. ———, and ———, Further experiments on the relation of vitamin B_1 to the growth of green plants. BOT. GAZ. **101**:491–500. 1939.
4. HAMNER, C. L., Effects of vitamin B_1 upon the development of some flowering plants. BOT. GAZ. **102**:156–168. 1940.
5. HITCHCOCK, A. E., and ZIMMERMAN, P. W., Further tests with vitamin B_1 on established plants and cuttings. Contrib. Boyce Thompson Inst. **12**:143–156. 1941.
6. MURNEEK, A. E., Vitamin B_1 vs. organic matter for plant growth. Proc. Amer. Soc. Hort. Sci. **38**:715–717. 1940.
7. SCHWARTZE, C. D., Western Washington Experiment Station, Puyallup, Wash. Private communication. 1940.
8. WENT, F. W., The air-conditioned greenhouses at the California Institute of Technology. In press.

RELATIONSHIP OF DISSOCIATION OF CELLULAR PROTEINS BY INCIPIENT DROUGHT TO PHYSIOLOGICAL PROCESSES

HENRY T. NORTHEN[1]

(WITH ONE FIGURE)

Introduction

When cellular proteins are subjected to environmental changes, the native proteins may be changed into labile ones which may be dissociated, associated, or denatured (27). The data presented here indicate that incipient drought, an experience common to plants, conditions a dissociation of protoplasmic proteins. This generally results in decreased resistance to lethal agents, increased protoplasmic swelling pressure, accelerated rates of respiration and polysaccharide hydrolysis, and may at times lead to mitotic abnormalities (27).

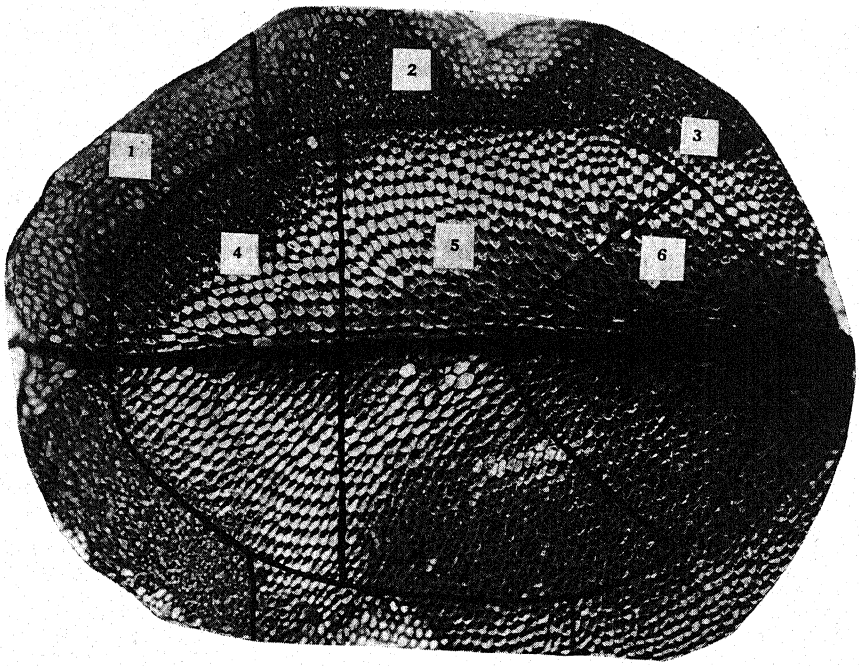


FIG. 1.—Fourth leaf, dried 20 minutes. Controls exhibited no displacement

MATERIAL AND METHODS.—Gametophytes of mosses, *Mnium* sp. and *Bryum* sp., were placed in a desiccator over anhydrous calcium sulphate. After exposures of 10–50 minutes, the plants were removed and centrifuged with an acceleration of 680× gravity for 3 minutes, a centrifugation which never displaced the chloroplasts in leaf cells of control plants. Next the approximate

[1] Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming, no. 192.

percentages of cells in which the chloroplasts were displaced were recorded for various zones (fig. 1) of the first sixteen leaves, the leaf nearest the stem tip being designated 1, the next 2, and so on. In other experiments with *Mnium* the gametophytes were dried for 2 hours, by which time they were completely shriveled. They were then transferred to water and centrifuged after various periods. Data were obtained as previously described.

TABLE 1

EFFECTS OF DROUGHT ON STRUCTURAL VISCOSITY OF PROTOPLASM

LEAF NUMBER	MINUTES IN DESICCATOR	PERCENTAGE OF DISPLACEMENT IN ZONE					
		1	2	3	4	5	6
4	*....						
	10....	100	0	0	75	0	0
	20....	0	0	100	100	100	40
	30....	0	0	0	0	75	100
	40....	0	0	0	0	0	0
	50....	0	0	0	0	0	0
8	10....	0	0	0	0	0	0
	20....	100	100	25	100	100	100
	30....	0	0	100	0	25	100
	40....	0	0	0	0	0	0
	50....	0	0	0	0	0	0
12	10....	0	0	0	0	0	0
	20....	0	50	75	25	50	100
	30....	75	100	75	100	100	100
	40....	0	50	100	100	100	100
	50....	0	0	0	0	0	0

* Controls: 0% in all zones of all leaves.

Data

The data for only the fourth, eighth, and twelfth leaves for one of eight according experiments with *Mnium* are recorded in table 1. As water is evaporated, the percentages of cells in which the chloroplasts were displaced become greater, but with further loss the percentages decrease. Hence during the initial stages of drought the structural viscosity of the protoplasm decreases, whereas in more advanced stages it increases. Whether the protoplasmic viscosity becomes greater than normal could not be determined, because the highest centrifugal acceleration available, 1062× gravity, would not displace the chloroplasts in control cells. However, FRITSCH and HAINES (8) and NORTHEN (25) previously noted that prolonged drought conditioned a higher-than-normal protoplasmic viscosity. It is probable that decreases in viscosity are conditioned in part by dissociations of protein molecules (splitting into molecules of smaller size), and that increases result from subsequent associations—that is, aggregation into molecules of greater size (27).

TABLE 2

EFFECTS OF PREVIOUS DROUGHT ON STRUCTURAL VISCOSITY OF PROTOPLASM

LEAF NUMBER	MINUTES IN WATER AFTER DRYING	PERCENTAGE OF DISPLACEMENT IN ZONE					
		1	2	3	4	5	6
4	*						
	15.....	0	0	0	0	0	50
	30.....	0	0	0	0	100	100
	60.....	0	0	0	0	0	0
	90.....	0	0	0	0	0	0
8	15.....	0	0	0	50	100	100
	30.....	0	0	0	100	100	0
	60.....	0	0	0	0	0	0
	90.....	0	0	0	0	0	0
12	15.....	0	0	75	100	100	100
	30.....	0	0	0	100	100	100
	60.....	0	0	0	0	0	0
	90.....	0	0	0	0	0	0

* Controls: (1) leaves immersed in water but not previously dried; 0% displacement in all zones of all leaves; (2) leaves after 2 hours of drying; 0% displacement in all zones of all leaves.

Abridged data for recovery experiments with *Mnium* are recorded in table 2. As evidenced by greater percentages of displacement than in controls, the pro-

teins become dissociated during the recovery period.

When gametophytes of *Bryum* sp. were subjected to drought, the results (a decreased protoplasmic viscosity followed by an increase to at least normal) were similar to those obtained with *Mnium*.

Discussion

Because the viscosity of many hydrophilic and hydrophobic sols *in vitro* does not decrease but rather increases as the concentration is augmented (20), the data support the previously advanced suggestion (27) that decreases in protoplasmic viscosity are conditioned in part by dissociations (disaggregations) of protein molecules. The fact that some protein molecules *in vitro* may be dissociated by increasing the concentration also supports this view. For example, PEDERSEN (30) noted that protein molecules could be split into molecules one-eighth of the original size by increasing the concentration.

Agents which dissociate protoplasmic proteins often condition an increase in the rate of respiration (27). When dissociation occurs a number of linkages are broken, salt linkages, hydrogen bonds, and of particular importance, disulphide linkages. When the latter are broken, sulfhydryl groups, which are activators of several respiratory enzymes (9, 12, 32, 33), are formed. Because with few exceptions enzymes are proteins or breakdown products of proteins (40), it is possible that they will be disunited from their combination with other proteins when dissociation occurs. Furthermore, respiratory substrates may become disengaged as a result of dissociation. If it be true that agents which condition dissociation make circumstances more favorable for respiration through an increase in such respiratory activators as -SH groups and through the disengagement of respiratory enzymes and substrate molecules, incipient drought should condition an increase in the rate of respiration. During the initial stages of drought, respiration is often increased in plants (19, 24, 29, 36, 37) and at times in animals (4, 7, 17, 18, 35). The data of PALLADIN and SHELOUMOVA (29) show all the expected consequences. They noted that the intensity of respiration of drying potato tubers first increased but with further drying decreased. Apparently the increase was conditioned by dissociation and the decrease by association. Furthermore, dried tubers, which had a decreased rate, respired more rapidly than controls when they were supplied with moisture. It will be recalled that the proteins became dissociated when dried moss leaves were again supplied with moisture.

The structural alterations of protoplasm which condition an increased respiratory rate may also condition an increased rate of polysaccharide hydrolysis, and indeed at times this may be partly responsible for the accelerated respiration (27). It is significant that carbohydrases, like respiratory enzymes, are actuated by -SH groups (15, 21). In addition, dissociation may result in the disengagement of carbohydrases from other proteins. KURSSANOV (16) states that enzymes are partitioned in the cell between the structural elements and the aqueous solution. In solution the enzymes hydrolyze and, when adsorbed, synthesize. Furthermore, he believes that changes in the physico-chemical condition in the cell may modify the ratio. When protoplasmic proteins dissociate, carbohydrases may accumulate in the aqueous phase, thus conditioning hy-

drolysis. If it be true that the dissociation of cellular proteins makes conditions favorable for polysaccharide hydrolysis, incipient drought should condition an accelerated rate. Polysaccharide hydrolysis is accelerated by wilting (1, 10, 13, 23) and by plasmolysis (14), which, like incipient drought, conditions a dissociation of cellular proteins (26).

The dissociation of cellular proteins by drought or hypertonic solutions augments the swelling pressure of protoplasm (27). This may account in part for the anomalous osmotic behavior of various cells in hypertonic solutions, although in such instances increased permeability and the production of osmotically active substances are other factors. With *in vitro* protein systems, the dissociation of protein molecules, conditioned by an increase in concentration, results in an augmented osmotic pressure (30). With respect to animal cells, BELKIN and SHEAR (2) noted that viable spindle cells swelled more in hypertonic solutions than they did in hypotonic ones. SOLLMANN (38) reported that the intravenous injection of a hypertonic solution produced at first an increase in brain volume. COOKE (5), ORT and MARKOWITZ (28), and HEILBRUNN (11) noted that muscle tissues in hypertonic solutions behaved as though they had a higher osmotic pressure than the solutions for which they were isotonic. In hypertonic solutions erythrocytes often behave anomalously (6, 31, 34). ROEPKE and BALDES (34) suggested that such irregularity may be partly the result of a dissociation of hemoglobin molecules, an explanation in harmony with the concepts developed here. For plant cells, WELCH (42) noted that the margins and bases of leaves of *Bryophyllum* which had dried for 11 weeks contained greater percentages of water and hence were more turgid than leaves from well-watered plants. BUHMANN (3) observed, contrary to expectations, that 37.8 per cent of the epidermal cells of *Bergenia cordifolia* increased in size in hypertonic sucrose solutions. Apparently in such cells the proteins were dissociated, and as a result the protoplasm imbibed water, a consequence of the increased protoplasmic swelling pressure.

Mitotic abnormalities may result from osmotic dehydration (39, 41, 44) or incipient drought (22, 44). MILOVIDOV (22) noted that the evaporation of water from root tips conditioned a dissolution of the phragmoplasts, and as a consequence cells with more than one nucleus were produced. Subsequent nuclear fusions resulted in the production of polyploid nuclei. The dissolution of the phragmoplasts probably resulted from dissociation and subsequent swelling. With respect to the formation of polyploid nuclei, it is significant that the widely used colchicine, like incipient drought, conditions a dissociation of cellular proteins—as evidenced by decreases in protoplasmic viscosity (43).

Summary

1. The centrifuge method was used to determine the structural viscosity of protoplasm in leaf cells of *Mnium* sp. and *Bryum* sp. which had been dried for periods up to 50 minutes. In other experiments with *Mnium*, dried plants were placed in water and the viscosity determined after periods varying from 15 to 90 minutes.

2. Incipient drought conditioned a decrease in the structural viscosity as a consequence of protein dissociations. During the recovery period a decrease in viscosity preceded the return to normal.

3. It is suggested that the dissociation of cellular proteins by incipient drought conditions an increased protoplasmic swelling pressure, accelerated rates of respiration and polysaccharide hydrolysis, and may at times lead to mitotic abnormalities.

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LITERATURE CITED

1. AHRNS, W., Weitere Untersuchungen über die Abhändigkeit des gegenseitigen Mangenverhaltnisse der Kohlenhydrate im Laubblatt von Wassergehalt. Bot. Archiv. 5:234–259. 1924.
2. BELKIN, M., and SHEAR, M. J., Chemical studies on tumor tissue. VI. Comparative effects of serum colloids and salts on the swelling of mouse tumor cells *in vitro*. Amer. Jour. Cancer 32:62–75. 1938.
3. BUHMANN, ANNIE, Untersuchungen Epidermiszellen von *Bergenia cordifolia*. Ein beitrag zur Fraze der Volumkontraktion der Zellen bei Plasmolyse. Protoplasma 27:114–124. 1936.
4. CALDWELL, G. T., Studies in water metabolism of the cat. The influence of dehydration on blood concentration, thermoregulation, respiratory exchange, and metabolic water production. Physiol. Zoöl. 4:324–359. 1931.
5. COOKE, ELIZABETH, Osmotic properties of frog's muscle. Jour. Physiol. 23:137–149. 1898.
6. DAVSON, H., Studies on the permeability of erythrocytes. III. The cation content of erythrocytes of rabbit's blood in hyper- and hypotonic sera. Biochem. Jour. 30:391–393. 1936.
7. DURYEE, W. R., The relationship between the water content and oxygen consumption of the organism. Science n.s. 75:520. 1932.
8. FRITSCH, F. E., and HAINES, F. M., The moisture relations of terrestrial algae. II. The changes during exposure to drought and treatment with hypertonic solutions. Ann. Bot. 37: 683–728. 1923.
9. GILL, P. M., and LEHMANN, H., Some factors influencing the formation of Robison ester from glycogen and inorganic phosphate in muscle extract. Biochem. Jour. 33:1151–1170. 1939.
10. GOLOVINA, A. S., The effect of drought on the biochemical processes of plants. Voronezh. Gosudarst Univ., Nauch. Raboty Studentov 1939: 51–58. 1939. (Chem. Abst. 36:4546. 1942.)
11. HEILBRUNN, L. V., The action of calcium on muscle protoplasm. Physiol. Zoöl. 13:88–94. 1940.
12. HOPKINS, F. G., MORGAN, E. J., and LUTWAK-MANN, C., The influence of thiol groups in the activity of dehydrogenases. II. Biochem. Jour. 32:1829–1847. 1938.
13. HOWARD, W. L., Physiological changes accompanying breaking of the rest period. Missouri Agr. Exp. Sta. Res. Bull. 21. 1915.
14. ILJIN, W. S., Die Permeabilität des Plasmas für Salze und die Anatonose. Studies Plant Physiol. Lab. Charles Univ. Prague 1:97–119. 1923. (Bot. Abst. 14:2941. 1925.)
15. ITO, R., Effects of reduction and oxidation on the reversible action of α-amylase, β-amylase, amylosynthease, sucrase, and urease. Hukuoka Acta Med. 32:88. 1939. (Chem. Abst. 35:5917. 1941.)
16. KURSSANOV, A. L., Untersuchungen enzymatischer prozesse in der Lebenden Pflanze. Advances Enzymol. 1:329–370. 1941.
17. LOEB, J., Further investigations concerning the necessity of free oxygen for the stimulation of development by hypertonic solutions. Arch. Ges. Physiol. (Pflüger) 118:30–35. 1907.
18. LOEB, J., and WASTENEYS, H., The influence of hypertonic solution upon the rate of oxidations in fertilized and unfertilized eggs. Jour. Biol. Chem. 14:469–480. 1913.
19. LUTHRA, J. C., Some experiments on the effects of dry and moist air on the rate of respiration and breakdown of ripe pears. New Phytol. 23:131–142. 1924.
20. MEYER, B. S., and ANDERSON, D. B., Plant physiology. New York. 1939.
21. MICHAELIS, L., and RUNNSTRÖM, J., Inactivation and regeneration of the glucolytic enzyme system of muscle extract. Proc. Soc. Exp. Biol. Med. 32:343–349. 1934.
22. MILOVIDOV, P. F., Durch Welken und Austrocknen künstlich hervorgerufene Mixoploidie bei Pflanzen. Protoplasma 30:427–459. 1938.
23. MOLISCH, R., Über den Einfluss der transpiration auf das Verschwinden der Stärke in den Blättern. Ber. deutsch. Bot. Ges. 39:339–344. 1921.
24. MOTHES, K., Die Wirkung des Wassermangels auf den Eiweissumsatz in höheren Pflanzen. Ber. deutsch. Bot. Ges. 46:59–67. 1928.
25. NORTHEN, H. T., Effect of drought on protoplasmic elasticity. Plant Physiol. 13:658–660. 1938.
26. ———, Studies on the protoplasmic nature of stimulation and anesthesia. Cytologia 10:105–112. 1939.
27. ———, Relationship of dissociation of cellular proteins by auxins to growth. BOT. GAZ. 103: 668–683. 1942.
28. ORT, F. M., and MARKOWITZ, J., Osmotic pres-

sure, bound water, and edema in perfused hearts. Amer. Jour. Physiol. **96**:541–546. 1931.

29. PALLADIN, W., and SHELOUMOVA, A. M., Effect of loss of water on respiration of plants. Bull. Acad. Sci. Russ., 6 series, no. 8. pp. 801–808. 1918. (Exp. Sta. Rec. **39**:731. 1918.)
30. PEDERSEN, K. O., Molecular state of proteins in mixtures and concentrated solutions. Nature (London) **138**:363. 1936.
31. PONDER, E., and SASLOV, G., The measurement of red cell volume. III. Alterations of cell volume in extremely hypnotic solutions. Jour. Physiol. **73**:267. 1931.
32. RAPKINE, L., Sulfhydryl groups and enzymic oxidation-reduction. Biochem. Jour. **32**:1729–1739. 1938.
33. RAPKINE, L., and TRPINAC, P., Role of sulfhydryl groups of proteins in the activity of dehydrogenases. Compt. rend. Soc. Biol. **130**:1516–1518. 1939.
34. ROEPKE, R. R., and BALDES, E. J., A study of the osmotic properties of erythrocytes. Jour. Cell. Comp. Physiol. **20**:71–93. 1942.
35. SCHIPPER, A. L., Some effects of hypertonic solutions upon development and oxygen consumption. Physiol. Zoöl. **11**:40–53. 1938.
36. SCHNEIDER, G. W., and CHILDERS, N. F., Influence of soil moisture on photosynthesis, respiration, and transpiration of apple leaves. Plant Physiol. **16**:565–583. 1941.
37. SMITH, A. M., The respiration of partly dried plant organs. Exp. Sta. Rec. **36**:824. 1917.
38. SOLLMANN, T., A manual of pharmacology. Philadelphia. 1942.
39. STROHMEYER, G., Beiträge zur experimentellen Zytologie. Planta **24**:470–509. 1935.
40. TAUBER, H., The chemical nature of enzymes. Chem. Revs. **15**:99–121. 1934.
41. WADA, B., Mikrurgische Untersuchungen lebender Zellen in der Teilung. III. Cytologia **7**:198–212. 1936.
42. WELCH, W. B., Water relations in *Bryophyllum calycinum* subjected to severe drying. Plant Physiol. **13**:469–487. 1938.
43. YAMAHA, G., and UEDA, R., Ueber die Wirkung des Kochizine auf *Spirobyra*. Bot. und Zool. (Syokubutu Oyobi Dobutu) **8**(11):1709–1714. 1940. (Biol. Abst. **15**:16214. 1941.)
44. ZWEIBAUM, J., and SZEJNMAN, M., Binuclear cells in tissue cultures. Bull. Intern. Acad. Polonaise **1935B**:37–48. 1935. (Chem. Abst. **30**: 3447. 1936.)

DEFOLIATION OF TOMATO PLANT AS A RESPONSE TO GASEOUS EMANATIONS FROM THE FRUIT[1]

JOHN SKOK[2]

Introduction

Defoliation of tomato plants during the latter part of the growing season is a major problem in eastern and midwestern tomato-producing areas. This response usually becomes evident when fruits start ripening and progresses rapidly as larger amounts of fruit are produced. It has been ascribed to a number of causes, the more important being leaf-spot diseases, unbalanced nutrition, and abnormal physiology (1, 5, 6, 7). The leaf-spot diseases have generally been regarded as the main cause, although spraying with fungicides seldom entirely prevents defoliation, and in many cases the effect is slight (5, 6).

ANDRUS *et al.* (1) and HORSFALL and HEUBERGER (5) have pointed out that fruit-load is concerned with susceptibility to defoliation, and SAMSON (8) states that defoliation coincides with the period of "heavy draft of the fruit on plant food resources."

A number of preliminary experiments and observations suggested that defoliation is in part a response to emanations of ethylene or of a combination of ethylene and other unsaturated hydrocarbon gases from ripe fruits underneath the vines. Minute traces of ethylene are present in the gaseous emanations from various plant tissues (3, 4), especially ripening fruits. The tomato plant is extremely sensitive to ethylene (2).

[1] These investigations are being conducted under a grant from Libby, McNeill, and Libby to the Department of Horticulture of the University of Illinois.

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Observations

Defoliation usually starts with shedding of those leaves nearest the crown of the plant in close proximity to the first ripened fruits. Progressive defoliation proceeds toward the terminal ends of the branches as subsequent clusters of fruits ripen. After defoliation has become extreme, only the leaves at the tips of the branches remain, that is, those which are beyond the clusters of ripe fruits.

Tomato fields almost bare of green leaves after severe defoliation often become green again in the latter part of the growing season, owing to new shoot growth in the axils of the leaf scars on the old vines. By that time most of the fruits have decayed or dried up, and apparently new growth is possible as soon as they are eliminated, weather conditions permitting. Those vines which fail to produce new shoots still have ripe fruits on them or in close proximity. The supply of nutrients and moisture is ample for growth.

During the last summer it was observed that plants grown in a number of home gardens generally failed to defoliate to the same extent as those in large commercial fields. From such garden plants the fruits were removed promptly upon ripening, whereas the fruits in the large fields were not always picked at the proper time; large quantities of over-ripe fruits accumulated, and the plants became defoliated.

Experimentation

These various observations suggested a possible relationship between fruit ripen-

ing, gaseous emanations from such ripe fruits, and defoliation. Experiments were devised which would test the hypothesis.

EXPERIMENT 1.—Young potted tomato plants placed in glass-inclosed chambers together with a number of ripe tomato fruits showed epinasty which became very severe after 18 hours. Induced epinasty by emanations from ripe fruits is probably mainly due to the presence of ethylene (2, 3). Two days after the plants had been placed in the chambers a number of the lower leaves abscissed, and on the fourth day only the top leaves remained, and these showed pronounced epinasty. On the sixth day most of the treated plants were dead. Control plants in similar chambers containing no ripe fruits showed none of these changes.

EXPERIMENT 2.—Late in August a number of young potted tomato plants were transplanted at random in a field of Early Baltimore tomatoes which were in full bearing and showed marked defoliation. The young plants were set near the old ones and so arranged that a number of ripe fruits were directly underneath them. A control group of young plants of the same lot was transplanted in the open at a distance of about 20 feet from the edge of the field and not near any ripening fruit.

After 4 days the young plants set close to the bearing plants had become pale green and some of their leaves and leaflets were curled, although there was no pronounced epinastic response. On the fifth day after transplanting the lower leaves were distinctly yellowed and curled, and the tips of several leaflets had dried. The following day or the day after, all the lower leaves died and dried up; the upper leaves were curled and had developed dried tips and edges. Some of the older leaves had abscissed, but in most cases the blades were dry and the petioles remained attached for a time and abscissed later. This curling, drying, and abscission is very similar in appearance to the usual defoliation which takes place in older fruit-bearing plants.

About 15–16 days after transplanting, the remaining upper leaves of the young plants in the field, which had been pale, curled, and dried along the tips and edges, seemed to regain color and resume growth, and the plants grew considerably in height in the next day or two. The fruits underneath these plants had dried by this time. Additional fresh fruits were placed there, but no apparent response was noticeable. The new leaves of the plants were 12–18 inches above the fruits at this time.

In addition to curling, drying, and abscission of leaves, spotting of the leaves also developed. These spots were present on the upper curled leaves as well as on the lower dried ones but were entirely absent from any leaves of the control plants. Microscopic examination of these areas showed the presence of spores of both *Alternaria* and *Septoria*. The presence of fruit emanations may have some effect in increasing the susceptibility of the tomato plant to these pathogens.

During the entire period the young control plants adjacent to the field remained green, vigorous, and at the end of the experiment had attained at least twice the size of the plants in the field. Their leaves were free from curling, and even the lowermost leaves had a bright color.

EXPERIMENT 3.—A number of plants of Early Baltimore and an unnamed determinate variety developed at the Station growing in a nearby garden were selected. The fruits of these plants had been harvested regularly at the market-ripe stage shortly after turning red. Al-

though the plants had produced an abundance of fruits, no defoliation had taken place as late as September 1, even on the determinate variety—which is ordinarily particularly subject to severe defoliation. In contrast to these plants, general defoliation was very pronounced in most commercial fields at this time. Ripe fruits were placed underneath the undefoliated vines of several plants of each of the varieties mentioned, and suitable adjacent controls were arranged. Eight days later defoliation was prevalent in all plants under which fruits had been placed but was more pronounced for the determinate variety than for Early Baltimore. More fruits were placed under the plants, and in another week defoliation had become extreme. All the leaves of both varieties had dried and abscissed, with the exception of a few at the tips of some of the branches which extended beyond the area where the ripe fruit had been placed. The plants about which no fruits had been placed remained green and did not defoliate.

Ripe fruits were also placed underneath the foliage of a so-called "bull" plant, a type of plant which is present occasionally in most large tomato fields. Such plants are usually large and vigorous, seldom produce any fruit, and do not lose their leaves even though defoliation is pronounced for other plants of the field. In the case of such vegetative, nonfruiting plants, 3 days after the fruits had been placed about them several of the leaves were yellow and curled, and at the end of 6 days a considerable number were dry. Additional ripe fruits were added later, and after about 2 weeks had elapsed practically all the leaves on the lower half of the plant had dried and abscissed. The upper leaves on the upright branches, which were considerably taller than those of normal-bearing plants, were the only ones which remained green. The leaves on this plant also had spots from which spores of *Alternaria* and *Septoria* were obtained. All other such vegetative nonfruiting plants of the field were examined and found to be entirely free of defoliation. Usually some of the leaves of these plants had a few *Alternaria* lesions, but the leaves were not discolored and the plants as a whole were free from either disease.

EXPERIMENT 4.—A number of plants in the field were stripped of all green fruits before the initiation of defoliation. These plants remained green, did not defoliate, and were relatively free from disease lesions, while adjacent plants permitted to bear fruit showed marked defoliation.

OTHER TESTS.—From the many observations it did not appear that the typical defoliation taking place in fields was brought about by a decreased supply of nutrients in the soil during the latter part of the growing period. In one trial a number of plants were supplied with solutions containing soluble and readily available nitrogen, phosphorus, and potassium. These elements were supplied singly and in various combinations at regular intervals. The plants so treated defoliated in the same manner as untreated plants whenever ripe fruits were present.

In another nutrition experiment conducted in the greenhouse a number of tomato plants were grown in a quartz gravel culture and supplied with nutrient solutions in which the levels of nitrogen, phosphorus, and sulphur varied. Nutrient solutions were applied daily during the earlier part of the experiment, followed by applications once each week, and still later once in 2 weeks. Finally nutrients were entirely replaced by daily watering. These treatments resulted in

varying responses in the different nutrient series, and after prolonged withholding of nutrients the plants in general turned pale and became hardened, but in no case was there any defoliation similar to that found in the field.

On the basis of the evidence collected to date, it is probable that gaseous emanations from ripe fruits present on the vines are a major contributing factor resulting in defoliation of tomato plants as grown under commercial field culture. On the basis of what is known about such emanations from ripening fruits of various kinds, and on additional experimental evidence, ethylene may be one of the principal compounds involved.

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LITERATURE CITED

1. ANDRUS, C. F., REYNARD, G. B., and WADE, B. L., Relative resistance of tomato varieties, selections and crosses to defoliation by *Alternaria* and *Stemphylium*. U.S.D.A. Circular 652. 1942.
2. CROCKER, WILLIAM, ZIMMERMAN, P. W., and HITCHCOCK, A. E., Ethylene-induced epinasty of leaves and the relation of gravity to it. Contr. Boyce Thompson Inst. 4:177–218. 1932.
3. DENNY, F. C., and MILLER, L. P., Production of ethylene by plant tissue as indicated by the epinastic responses of leaves. Contr. Boyce Thompson Inst. 7:97–102. 1935.
4. GANE, R., The formation of ethylene by plant tissues, and its significance in the ripening of fruit. Jour. Pom. Hort. Sci. 13:351–358. 1935.
5. HORSFALL, J. G., and HEUBERGER, J. W., Causes, effects and control of defoliation on tomatoes. Connecticut Agr. Exp. Sta. Bull. 456. 1942.
6. MACGILLIVRAY, J. H., Studies of tomato defoliation. Proc. Amer. Soc. Hort. Sci. 33:528–532. 1936.
7. MACGILLIVRAY, J. H., and SAMSON, R. W., Further studies of tomato defoliation. Proc. Amer. Soc. Hort. Sci. 34:523–526. 1937.
8. SAMSON, R. W., Defoliation of deflorated, late-set and vigorously fertilized tomato plants by *Septoria lycopersici*. Purdue Univ. Agr. Exp. Sta. Rept. 30:41–42. 1940.

VARIETAL DIFFERENCES IN ANATOMY OF CROSS-SECTION OF WHEAT GRAIN[1]

JAMES C. BATES

(WITH TEN FIGURES)

The varieties of wheat selected for this study were Turkey, Tenmarq, Blackhull, Chiefkan, and Kawvale. The grains were obtained from the Kansas Agricultural Experiment Station rust nurseries on June 4, 1941. Average-size grains were selected from the mid-portion of the spike. The grain was in the stiff-dough stage.

After shaving off the ends, the grains were treated for 48 hours with killing and fixing fluid (2 per cent chromic acid, 25 cc.; 10 per cent acetic acid, 25 cc.; commercial formaldehyde, 15 cc.; and distilled water, 25 cc.). The solution was then removed by washing the grains overnight in running water. After washing, they were treated for 2 hours in each of the following: dioxan-water, 1:2; dioxan-water, 1:1; dioxan-water, 2:1; and two changes of pure dioxan.

After dehydration, the material was transferred to a saturated solution of parawax in dioxan in the paraffin oven, 12 hours; two changes of pure parowax, 3 hours each; then transferred to Tissuemat (Fisher, M.P. 56–58) and cast into blocks. The blocks were left in water until time of sectioning. When difficulties were encountered, the paraffin was cut away at one end and the grain left in water for a few hours longer. The sectioning was done with a rotary microtome, using a safety razor blade. The paraffin ribbon was cut into pieces and floated on water at 35°–40° C. The pieces were then floated on clean slides previously treated with Szombathy's gelatin adhesive as modified by HAUPT,[2] and while still wet were exposed to the fumes of commercial formaldehyde for an hour or longer. They were then dried in the air, the paraffin removed with xylene, and mounted in hyrax without staining.

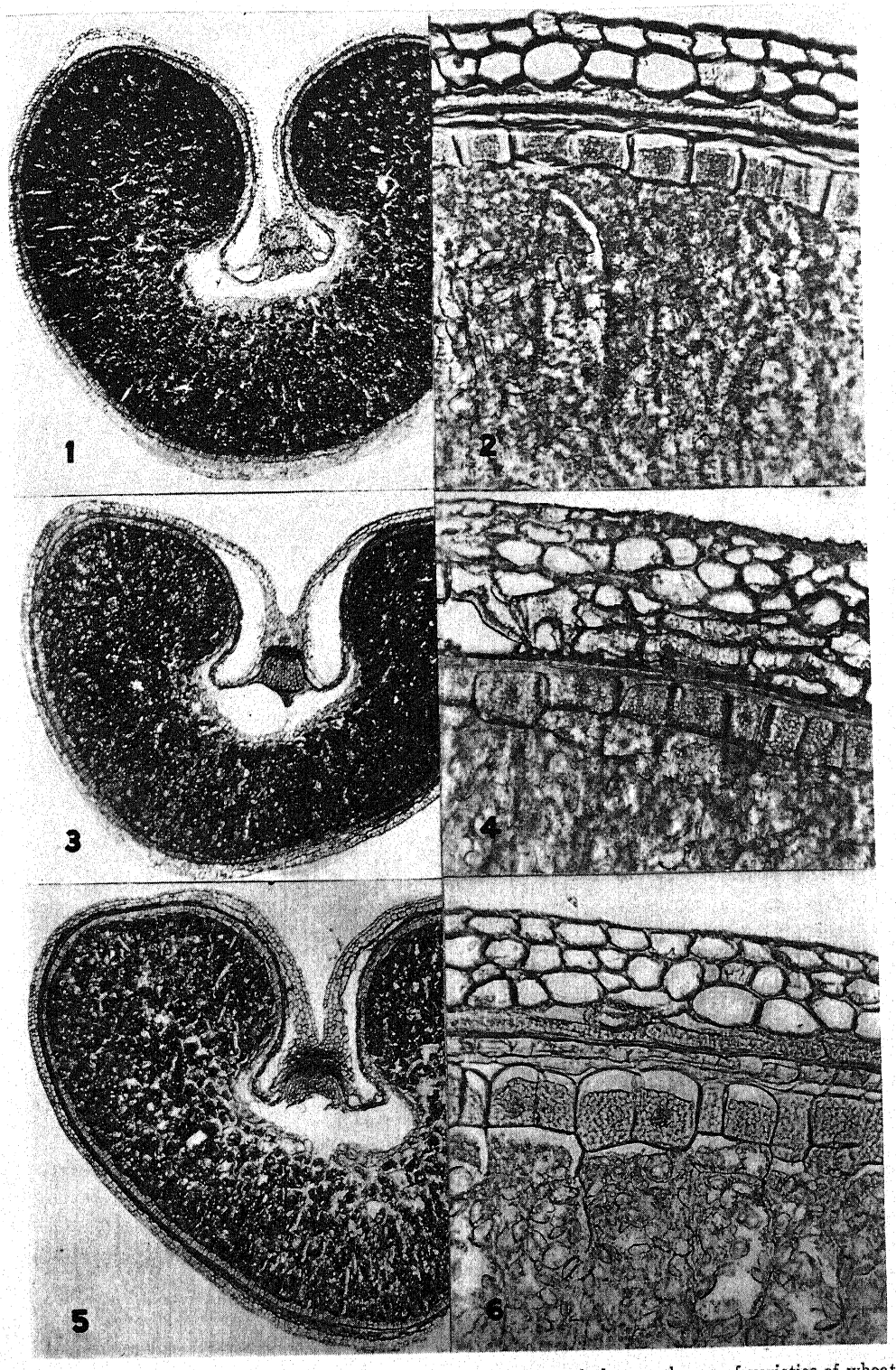
Figures 1–10 show the cross-sections and portions of the bran layer.

The data in table 1 were calculated from measurements taken from the photomicrographs. They show that although the cross-sectional area of the grain is less and the combined thickness of the bran and aleurone layers is less in Kawvale than in Tenmarq, the area of the combined bran and aleurone layers is greater and consequently the percentage cross-sectional area of starchy endosperm is less in Kawvale. This is due to the difference in the shape of the cross-section (figs. 1, 2, 9, 10).

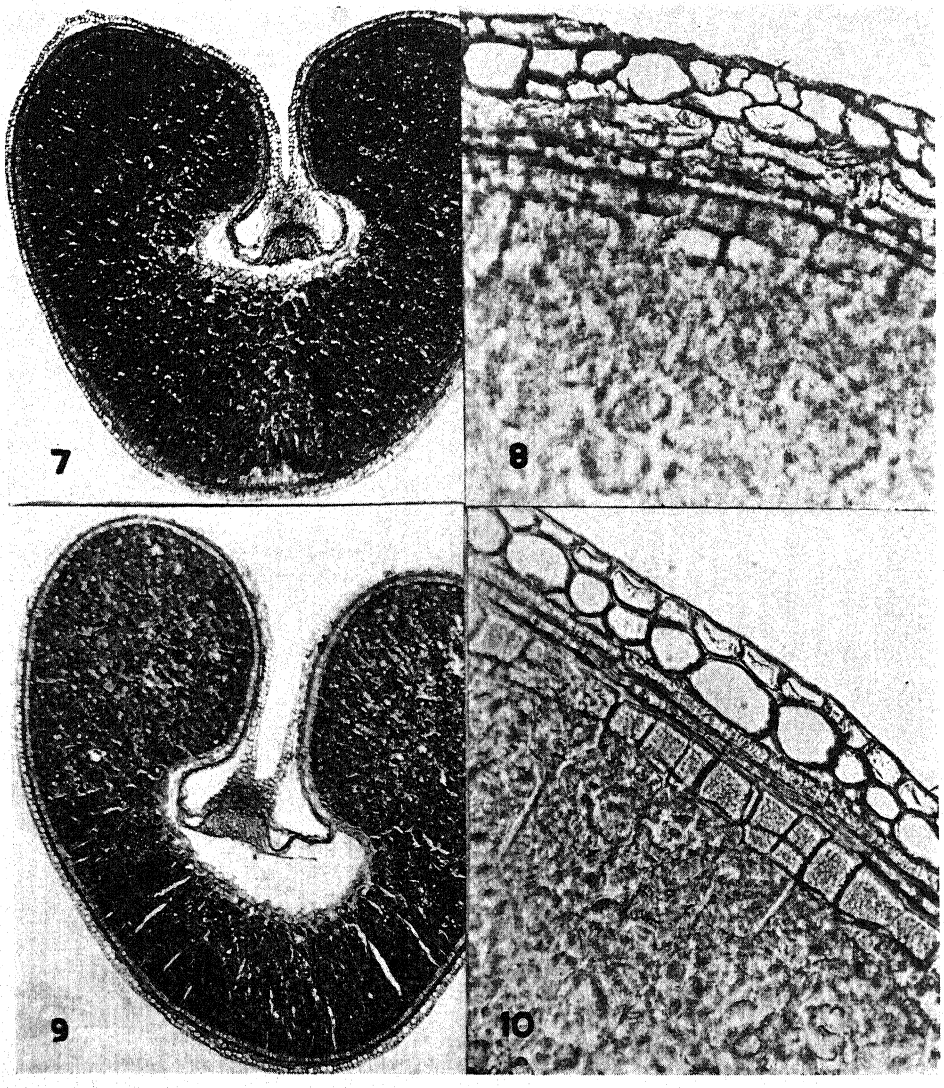
The combined thickness of the bran and aleurone layers is greater in Blackhull (figs. 3, 4) and Chiefkan (figs. 5, 6) than in the other varieties. The percentage cross-sectional area of starchy endosperm is smaller in Blackhull than in any other variety. Chiefkan has a larger cross-sectional area of grain and a larger percentage cross-sectional area of starchy endosperm than has Blackhull, although

[1] Contribution no. 434 from the Department of Botany, Kansas Agricultural Experiment Station.

[2] HAUPT, A. W., A gelatin fixative for paraffin sections. Stain Technol. 5:97–98. 1930.



FIGS. 1–6.—Cross-section of grain and section through bran and aleurone layers of varieties of wheat: figs. 1, 2, Tenmarq; figs. 3, 4, Blackhull; figs. 5, 6, Chiefkan.



FIGS. 7–10.—Cross-section of grain and section through bran and aleurone layers of varieties of wheat: figs. 7, 8, Turkey; figs. 9, 10, Kawvale.

the combined thickness of the bran and aleurone layers is about the same.

TABLE 1

ANATOMICAL DIFFERENCES IN CROSS-SECTION OF WHEAT GRAIN

VARIETY	CROSS-SECTIONAL AREA				COMBINED THICKNESS OF BRAN AND ALEURONE LAYERS (MM.)
	Grain (sq. mm.)	Bran and aleurone layers combined* (sq. mm.)	Starchy endosperm (sq. mm.)	Starchy endosperm (%)	
Hard red winter wheats					
Tenmarq....	5.91	1.32	4.59	77.66	0.106
Chiefkan....	5.96	1.71	4.25	71.30	0.143
Turkey.....	5.55	1.60	3.95	71.17	0.112
Blackhull....	4.53	1.37	3.16	69.60	0.140
Semi-hard red winter wheat					
Kawvale....	5.71	1.50	4.21	73.73	0.097

* Areas of large intercellular spaces at base of crease excluded.

Turkey (figs. 7, 8), which has frequently been used as a standard in testing wheat varieties, comes nearest to representing the average grain in this study of varietal differences.

The percentage volume of starchy endosperm which greatly influences percentage flour extraction is determined by the size and shape of the grain, combined thickness of bran and aleurone layer, and proportionate size of the germ. Since the percentage cross-sectional area of starchy endosperm is determined by the shape, area, and combined thickness of bran and aleurone layers in the cross-section, it is undoubtedly an important factor in determining the percentage of flour extraction.

Appreciation is expressed to Dr. JOHN H. PARKER, Director of the Kansas Wheat Improvement Association, for suggesting this problem.

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CURRENT LITERATURE

Upper Cretaceous Floras of the Rocky Mountain Region. II: Flora of the Lance Formation at Its Type Locality, Niobrara County, Wyoming. By ERLING DORF. Preprinted from Carnegie Institution of Washington Publication no. 508. October 2, 1942. Pp. 79+159. Illustrated.

The Lance flora, long a source of uncertainty concerning the Mesozoic-Cenozoic boundary, has been studied from abundant, new carefully made collections. Nearly all the earlier material was examined also, and it now seems evident that little conflict remains between age interpretations based on animal and plant fossils. Lancian age is defined as the temporal term for latest Cretaceous of the Rocky Mountain region, delimited below by true Fox Hills and above by beginning of the Paleocene.

The seventy species of plant fossils assembled from the Lance differ considerably from those known in older beds and contrast more strongly with assemblages of the early Mesozoic. At the same time all but thirteen of the Lancian species are known to occur in other latest Cretaceous strata outside the type district of the Lance formation, so that the flora affords substantial practical assistance to the geologist. The plant assemblages are ably summarized in tables and discussion. Dominant forms are chiefly dicots but include abundant *Sequoia* and *Araucarites*. Analysis suggests that the flora existed under warm temperate more nearly than under subtropical conditions in humid lowlands.

In discussing the principles and methods of correlation, DORF mentions similarity of assemblages, use of widespread short-ranging "index" fossils, and use of a widespread dominant form or forms not necessarily of restricted age. The earliest appearance of new vegetational types is preferred to the latest known occurrence of old as a datum for correlation purposes. Correlation based on the recognition of evolutionary series is not mentioned, although in other paleontological investigations the method has been particularly constructive, since it provides a more adequate explanation for the appearance or disappearance of biologic entities.

Of the seventy forms treated, forty-nine have been referred to thirty-three modern plant families, one only questionably. Fifteen modern genera, four of them questionably, are included under their respective families. Forty-seven forms are apparently generically distinct or else their correlative modern genus has not been recognized.

Probably all, or nearly all, scientists will admit the desirability of identifying modern plant groups as far back in the fossil record as they can be reliably traced. DORF has made extensive comparisons of his fossils with modern specimens, and apparently all allocations to modern genera have been made with careful discrimination. Many of these allocations, however, do not accord with the current revised code of nomenclature (1930 Cambridge revision; Gustav Fischer, Jena, 1935), which provides (p. 4) that "the nomenclature of Fossil Plants of all groups begins with the year 1820." Obviously the types of modern genera validly established before that date cannot be utilized for identification of fossils if these rules are to be followed. Needless to say this addition to Article 20 has not increased the prestige of formalized nomenclature among paleobotanists. DORF's study of the Lance flora, together with many other of the better treatments of Cretaceous and Tertiary fossils, illustrates the desirability of modifying this regulation.—JAMES M. SCHOPF.

The Carnivorous Plants. By FRANCES ERNEST LLOYD. Waltham, Massachusetts: Chronica Botanica Co.; New York City: Stechert and Co., 1942. Pp. xv+352. Figs. 556.

Biologists will welcome this interesting and comprehensive book. Thirteen chapters are given to the fifteen genera of carnivorous angiosperms. For each there is discussion of its discovery, habitat, distribution, vegetative organization, propagation, and seed formation. An additional chapter takes up certain fungi for which zoöpahgy is known. A bibliography concludes each chapter.

Special attention is given to the traps, as to type, structure, mechanism, and operation. The structural survey of these organs includes anatomical and developmental details as well as consideration of homologies. For each type there is discussion of its secretions and any experimental work relating to the nutritive utilization of the trap contents. Much information is brought together about trapped organisms and others commonly living in or associated with these structures.

All illustrations except a few text figures are grouped on thirty-eight plates, bound in the back of the book. These include both photographs and drawings, largely original, and most of them are by the author. Naturally the volume finds its climax in the chapters on *Utricularia* and related genera, since they are enriched by the author's extended researches on these forms. His critical judgment, however, has been extended to all parts of the book and becomes its strongest recommendation to botanists. —ROBERT B. WYLIE.

Fieldbook of Native Illinois Shrubs. By LEO R. TEHON. Urbana: Illinois Natural History Survey Division, Manual 3, 1942. Pp. vi+307. Illustrated. $1.25.

This book is a complement to the previously published manual describing the wild flowers of Illinois. Native and a few naturalized shrubs, including the American yew, the common juniper, several species of *Smilax*, and species belonging to forty dicotyledonous families are keyed and described. Line drawings and four color plates illustrate certain features of every species. An introductory discussion of habitats, a map of Illinois, a glossary and an index complete the book. Its usefulness will undoubtedly extend far beyond the boundaries of Illinois.—PAUL D. VOTH.

EFFECTS OF MICRO-NUTRIENT DEFICIENCIES ON GROWTH AND VITAMIN CONTENT OF THE TOMATO

C. B. LYON,[1] K. C. BEESON,[2] AND G. H. ELLIS[3]

Introduction

The nutritive values of food plants and the factors which may modify them are subjects of increasing and timely importance. During the last two summers, extensive experiments have been conducted at this laboratory on the effects of varying concentrations of macro-nutrient elements in the nutrient medium on the vitamin content of tomato fruits and turnip greens (8, 19, 20). A survey of the effects of micro-nutrient deficiencies on the vitamin content and mineral composition of tomatoes is reported in this paper.

Micro-nutrient elements, that is, those mineral elements essential in minute quantities for growth and reproduction of higher plants, have received much attention in recent years. The early research of MAZÉ (31, 32, 33) and others stimulated this interest, and the list of essential elements has been expanded to include manganese (34, 1), boron (49, 53), zinc (47, 49), copper (27, 48), and molybdenum (2, 4) and is probably now incomplete (26).

There have been several reports that various deficiencies of the micro-nutrients may influence vitamin content. POWERS (38) has stated that the addition of boron to soils deficient in this element resulted in a 30 per cent increase in the carotene content of alfalfa. ROY and BAHRT (40) have reported that oranges from zinc-deficient trees contain subnormal amounts of ascorbic acid and that the addition of zinc would restore the ascorbic acid to the normal level. According to HAMDALLAH (18), *Phaseolus multiflorus* or *Zea mays* grown under iron-deficient conditions contains as much or more ascorbic acid than normal plants. COWART (11) has reported that combined applications of copper, manganese, and zinc in sprays to citrus trees may result in greater ascorbic-acid content of the fruits.

Material and methods

Most recent methods of demonstrating deficiency effects of micro-nutrients in solution culture have involved growing plants to various stages of maturity using rigidly purified distilled water, salts free from undesired heavy metals (50, 51), aeration by sintered Pyrex-glass aerators, and Pyrex containers. Reasonable care has been exercised with other factors of the environment which might provide possible sources of contamination. There are two principal difficulties with this method. First, the volume of nutrient supply has been relatively small. It is necessary to change the nutrient medium frequently or to replenish it with ions which have been depleted as a result of plant growth. This is especially true when it is desirable to grow plants to maturity. Second, the aerators have been subject to clogging from algae, dust, or other particles. It is also difficult to se-

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cure uniform aeration in replicated cultures. An apparatus designed especially for nutritional studies in the greenhouse eliminates many of these difficulties (fig. 1).

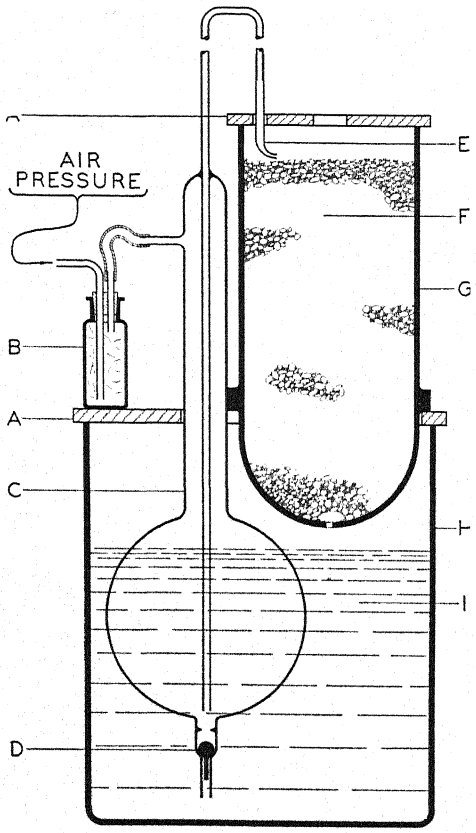


Fig. 1 (*left*)

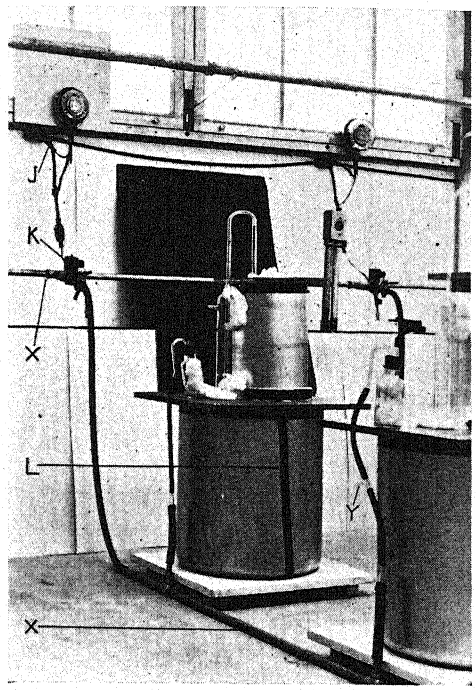


Fig. 1 (*right*)

FIG. 1.—Left, diagram of Pyrex-glass apparatus used to grow tomato plants to maturity using nutrient solutions deficient in micro-nutrient elements. Right, arrangement of apparatus in greenhouse: *A*, $\frac{3}{4}$″ plyboard; *B*, 200-cc. wide-mouthed bottle filled with cotton; *C*, trap; *D*, Pyrex-glass valve; *E*, Pyrex-glass delivery tube; *F*, Pyrex gravel; *G*, round-bottomed Pyrex jar; *H*, Pyrex jar; *I*, nutrient solution; *J*, General Electric time switch 86×843; *K*, electrically controlled solenoid valve; *L*, black scotch tape; *X*, $\frac{3}{8}$″ air line of galvanized iron pipe; *Y*, T-tube with exposed arm made from capillary tubing.

The apparatus consisted of a round, flat-bottomed Pyrex jar (*H*) 16 inches in diameter and 16 inches high, with a capacity of 39 liters of nutrient solution when filled to within 4–5 inches of the top. The jar was first painted on the outside with black enameloid paint to exclude light, and over it a second coat of aluminum paint was applied to prevent excessive heating of the nutrient solution. A strip of black scotch tape (*L*) covered an unpainted area and could be removed when necessary to examine the level of the solution. Three-fourths inch plyboard (*A*) was placed over the top of the jar and painted with three coats of Vinylite plastic paint.[4] Condensation of moisture occurred on the plyboard, and in so far as could be determined the plastic paint was inert and did not contribute any ions to the cultures. The plyboard in

[4] Obtained from Barrett Varnish Company, Cicero, Illinois (No. 5003 clear Vinylite solutions).

turn supported a second round-bottomed Pyrex jar (*G*) with a $\frac{1}{4}$-inch hole at the bottom to permit draining of the nutrient back to the reservoir. These jars were especially constructed with a glass flange approximately 6 inches above the bottom to act as support. They were painted as in (*H*) above. The jars were filled with Pyrex gravel, which consisted of crushed glass from colloidal to walnut sizes. The trap (*C*) was made from 51-mm. outside-diameter Pyrex glass tubing which was sealed to a 5-liter boiling flask. The inside delivery tube was made from 8-mm. Pyrex glass tubing, which was widened at the mouth (*E*) to deliver a sheet of solution at the surface of the Pyrex gravel. A valve (*D*) was sealed in at the bottom of the 5-liter boiling flask.

The seedlings were inserted in the Pyrex gravel substrate (*F*) with the top of the plant protruding through the plyboard (*A*). Cotton was inserted between the stem of the plant and the plyboard and between the plyboard and delivery tube (*E*). Cotton was also inserted in all openings of the plyboard (*A*) which supported the trap (*C*) and jar (*G*). Thus the interior of the apparatus was protected from possible dust and other impurities from the external atmosphere.

A time clock (*J*), operated so that current could be turned on for any desired portion of its complete cycle of 30 minutes, was inserted in the air line (*X*) leading from the compressor to the apparatus. For our purpose, the clock was so regulated that the current was turned on for 15 seconds in each 30-minute cycle. The current opened the solenoid valve (*K*)[5] to admit air pressure, which was filtered to the apparatus through the cotton at (*B*).

[5] Obtained from Minneapolis-Honeywell Regulator Company, Minneapolis, Minnesota (valve No. V437A).

Thus, once every half hour for the duration of the experiment, air pressure was admitted to the apparatus. The valve (*D*) was seated and the nutrient within the trap passed through the delivery tube (*E*) to the surface of the Pyrex gravel. The nutrient, the quantity of which was controlled by regulating the duration of the flushing period, percolated through the gravel and was returned to the reservoir (*H*). When the air pressure was turned off at the end of the flushing interval, the capillary tubes (*Y*) in the air line expelled air until the pressure within the apparatus was equalized with atmospheric pressure. The pressure of the nutrient (*I*) then forced the valve (*D*) open and the trap refilled with the nutrient.

Such an apparatus provides adequate supply of nutrient for large plants and does not require frequent replacement of solutions. Automatic aeration is provided by the frequent percolation of the nutrient through the substrate. The apparatus may be arranged in any desired experimental design, but one entire replication (consisting of a variable number of cultures) must be supplied with a common air line, solenoid valve, and time clock, thus providing all cultures with the same volume of nutrient during a flushing interval. Water was purified in 12-gallon Pyrex-glass carboys by filling them with distilled water and 100 gm. $CaCO_3$, 100 cc. M/1 K_2HPO_4, and 50 cc. M/1 $Ca(NO_3)_2$. A Pyrex-glass tube with a cotton filter was connected to an air line and extended to the bottom of the carboy. Air was bubbled through the mixture at a sufficient rate so that the $CaCO_3$ and the precipitate formed by the addition of $Ca(NO_3)_2$ and K_2HPO_4 were continually agitated throughout the mixture. Continued agitation was necessary for a period of 8 hours, and the adsorbents were allowed to settle for 36 hours.

The supernatant water, free from heavy metals, is siphoned off through Pyrex tubing. The carboy may be refilled with water without removal of the adsorbents. For each subsequent purification it is necessary to add 100 cc. M/1 K_2HPO_4, 50 cc. M/1 $Ca(NO_3)_2$, and 50 gm. $CaCO_3$. All purified water was tested by the dithizone technique. It was possible to purify 250 gallons in 24 carboys during each 72-hour period.

For the control plants, 39 liters of nutrient solution were used in each culture. The following quantities of nutrients were present in addition to a small quantity of those contributed by the purification process:

Ca	12 m.e./l.
Mg	9
K	9
NH_4	2
SO_4	9
HPO_4	11
NO_3	12
B (as H_3BO_3)	0.5 p.p.m.
Mn (as $MnCl_2 \cdot 4H_2O$)	0.5
Zn (as $ZnSO_4 \cdot 7H_2O$)	0.05
Cu (as $CuSO_4 \cdot 5H_2O$)	0.02
Mo (as 85% MoO_3)	0.05
Fe (as $FeCl_3$)	5.00

For the macro-nutrients it is necessary to purify only four salts, which were prepared as molar solutions, with appropriate dilutions when the nutrient medium was made up. The salts, together with their final molar concentrations, are as follows: $Ca(NO_3)_2$, 0.006 M; KH_2PO_4, 0.0045M; $MgSO_4$, 0.0045 M; $(NH_4)_2HPO_4$, 0.001 M. Methods developed by STEINBERG (50) and modified by STOUT and ARNON (51) were used for the purification of all salts containing macro-nutrient ions from heavy metals.

Since iron was the only micro-nutrient added in appreciable amounts to the nutrient medium, and since iron salts commonly contain varying quantities of copper and other elements, $FeCl_3$ was the only salt of a micro-nutrient which was purified. The procedure involved a repeated ether extraction of the iron from a hydrochloric-acid solution (25). If desirable, ferric chloride may also be separated from molybdenum by the basic acetate method (28).

The nutrient solution of any micro-nutrient deficient culture was prepared by adding to the basal nutrient of macro-elements all supplements of micro-nutrient elements, with the exception of the element under consideration. Water was added twice weekly to maintain a constant volume. At the same time the pH was adjusted to 5.5 with HNO_3 (14). During the last month of the experiment, when the plants were large, the nutrient was made up to volume with purified water three or four times weekly. When needed, additions of iron were made to the culture solutions.

Chemical methods were used for the determination of ascorbic acid, with a modification described by MORELL (36). The aliquot, however, was titrated to an end point with standardized dye. The method of provitamin-A determination is described by ELLIS *et al.* (16). It consists of an extraction of diacetone and acetone-soluble pigments, which are transferred to petroleum ether. A separation of the pigments is made on a Tswett column consisting of a mixture of magnesium oxide and Supercell. The top band of pigments (largely lycopene) is discarded and the lower band (largely carotene) is eluted with petroleum ether containing 4 per cent ethanol. The concentration of the pigment is determined in a photoelectric colorimeter previously calibrated with β-carotene. The method is somewhat empirical, but it determines a group of carotenoid pigments consisting mostly of β-carotene. Unpublished data

show good agreement with a rat bioassay on a sample of canned tomato puree. The method is rapid, permitting completion of the initial stages of the extraction and transfer of the pigments to petroleum ether within an hour after the fresh fruits are sampled. Riboflavin[6] was determined by SNELL and STRONG'S microbiological method (46).

Mineral determinations were made by the following methods: zinc, COWLING and MILLER (12); iron, SAYWELL and CUNNINGHAM (43); copper, COULSON (10) with modifications suggested by DRABKIN (13); molybdenum, MARMOY (30) with modifications suggested by ROGERS (39); boron, NAFTEL (37); and manganese according to the Official Method (5). The analyses were made on unwashed leaflets, since in many instances the leaflets were dead.

Experimentation and results

On April 20, 1942, inbred seeds of Bonny Best tomatoes were planted in large flats with specially prepared acid-washed sand and watered with purified water adjusted to a pH of 5.5 with HNO_3. This water contained only small quantities of ions contributed by those salts used in the purification process ($Ca[NO_3]_2$, K_2HPO_4, and $CaCO_3$). The seedlings were grown under these conditions until May 31, when they were approximately 3 inches tall, very chlorotic, and purple on the under side of each leaf. Deficiency symptoms of several macro-nutrients were clearly evident. By chemical analyses the seedlings were shown to be deficient with respect to micro-nutrients as well. The cotyledons were removed, uniform seedlings transplanted into the apparatus, and immediately supplied at $\frac{1}{2}$-hour intervals with their respective nutrient solution. The plants became normal in appearance within a period of 1 week, with a healthy green color in leaves and stems. As they developed, the plants were supported by cords suspended from the greenhouse roof, and all axillary growth was removed twice weekly. When all plants were harvested, October 1, the number of internodes was counted on each plant and the aerial vegetative growth divided into three parts, with an equal number of elongated internodes in each part. The expanded leaflets from each of these divisions were preserved for micro-nutrient analyses.

During the course of the experiment, each fruit was picked on the morning of the day that complete color change had occurred. On each plant the second, fourth, and sixth fruits to ripen were allocated for chemical analyses, while the first, third, fifth, and seventh fruits were used for vitamin determinations. When picked, the fruits for vitamin assays were immediately weighed and transverse sections weighing approximately 10 gm. taken from the equatorial portion.

Forty-nine cultures were used in a Latin square (17), with seven treatments and seven replications randomized by the use of TIPPETT'S tables (52). All data were reduced and analyzed by means of the analysis of variance (45), and the t test was used for determining significant differences. Odds as great or greater than 99:1 were accepted as proof that the observed differences were not due to the errors of random sampling. The nature of the results was such that it was impossible to maintain orthogonality. In such cases, only actual data were used in the analysis of variance, and a second analysis was computed using substituted values obtained by YATES'S method (54), with one degree of freedom deducted for each substituted value. In no case did

[6] The aid of Miss R. MACHATA in preparing and incubating bacterial cultures is appreciated.

the latter procedure change the conclusions that could be drawn by the former method.

Analyses of variance were computed for each character, and the results are compiled in table 1. It is evident that significant differences between treatments are inherent in the data for vegetative growth and fruit production, since all F values are well in excess of those required for a P value of 0.01. It is also evident that the effects of environment when measured by replication did not produce statistically significant differences in any of the characters used as criteria of growth and fruitfulness, except the dry weight of the vine. The differences in the environment measured in this experiment may be due to differences between replications resulting from minute variations in the amount of nutrient delivered during the flushing period or to differences in the position of the plants in the greenhouse. The latter possibility seems the more probable, and in this case the difference would result from variations in climatic environmental factors. In this connection, the data for fresh weight of vine showed no significant effects resulting from position in the greenhouse.

TABLE 1

RESULTS OF ANALYSES OF VARIANCE ON CHARACTERS FOR WHICH DATA WERE RECORDED

CHARACTERS	F VALUE	
	Between treatments	Between replications
Vegetative growth*		
Height of plants	26.45	0.57
No. of internodes	16.47	0.81
Fresh weight of vine	114.87	2.27
Dry weight of vine	63.58	4.08
Fruit production*		
Total fresh weight of fruit per plant	28.15	1.84
No. of fruit ripened per plant	19.66	1.34
No. of immature fruit per plant	24.75	2.27
Total no. of fruit per plant	37.68	1.32
Vitamin content of fruit†		
Ascorbic acid	8.13	1.77
Provitamin A	1.73	1.72
Riboflavin	3.64	11.80

* F values 3.47–3.56 required for P value of 0.01 for both statistics.

† F values 2.97–2.99 required for P value of 0.01 for both statistics.

It is evident that ascorbic-acid and riboflavin content were significantly influenced by treatment, while no significant difference between treatments was demonstrable with respect to provitamin A. Riboflavin was the only vitamin tested which was significantly influenced by position in the greenhouse. The possibility of the effects of variations in technique being confounded with the effects of plant position is not indicated with the number of analyses used for each vitamin (approximately 125).

Since significant effects with respect to treatment are shown in almost all the characters studied, data for vegetative growth and fruit production are given in table 2. The control plants were tall, thick-stemmed, and had healthy apical growing points. The leaves were dark green, fully expanded, and had a few extra leaflets which developed from buds at the junction of the leaflet and petiole. The fruit production of these plants was comparable in quantity with that produced by plants under optimum treatments of previous experiments (19), although mature fruit size was somewhat reduced. The fruits were firm and had normal colorations during ripening. Both leaflets and fruits contained normal quantities of micro-nutrients (7). During the early part of September, when the plants were large and had produced six mature fruits, the laminae of the lowermost leaves became chlorotic, a condition not curable by additions of iron, and there was some purple spotting. The chlorosis and purpling did not progress upward on the plants (figs. 2*F*, 3*A*). This condition sometimes occurs in old field-grown plants.

MANGANESE DEFICIENCY

The first symptom of manganese deficiency was a spotted chlorotic condition which appeared when the plants were 10 weeks old (4 weeks after transplanting). The leaflets at this time were not so large as the control leaflets, although the plants were growing vigorously. The chlorosis was first observable in the blades of the leaflets near the top of the plant but soon became general and was accompanied by a yellowing of the entire leaflet lamina. The leaflets became completely brown and subsequently developed necrosis at the tip or edges, or in some instances at the midvein of the leaflet (fig. 3*B*). When the plants were 19 weeks old, leaflets of the entire upper three-fourths of these plants appeared dead (fig. 2*C*). The stem and petioles began to develop intermittent brown spotting, and the top part of the stems began to turn yellow. At the conclusion of the experiment the top part of the stem was entirely brown and dried up. The final height of the manganese-deficient vines, as well as the fresh and dry weights, was significantly less than that of the control plants (table 2).

Fruit production was also significantly less in all characters used as criteria of fruitfulness, except for the mean weight of ripe fruits where statistically significant differences were not demonstrable. This deficiency was also evidenced by fruit symptoms. In normal ripening, the stem end of the fruits is the last portion to become completely red. This is also true of the manganese-deficient fruits, but the fruits were somewhat more yellow than normal ones during all stages of development until ripening occurred. At the stem-end portion, yellow-green areas persisted during ripening and never became red. In these green areas pin points of brown occurred at random. The brown spots may occur prior to the time that the fruits start to become red, although more commonly they occurred subsequent to the onset of ripening. One manganese-deficient fruit was allowed to remain on the vine until it had completely dried up. The green areas remained clearly evident through the soft-ripe stage and during the drying process.

Replicates of the manganese-deficient treatment showed to varying degrees the symptoms just mentioned. Chemical an-

TABLE 2

GROWTH MEASUREMENTS AND FRUIT PRODUCTION, GIVING TREATMENT MEANS TOGETHER WITH THEIR STANDARD ERRORS

CHARACTERS	CONTROL PLANTS	DEFICIENT TREATMENT					
		Mn	Zn	Mo	Cu	B	Fe
Vegetative growth							
Height of plants (cm.)	307± 5.6	179± 12.6	239 ± 18.4	299± 9.4	163± 10.7	291± 13.1	172± 14.5
No. of internodes	44± 1.2	29± 1.3	36± 2.9	42± 1.8	28± 1.0	42± 1.1	32± 0.8
Fresh weight of vine (gm.)	2293± 48.5	685± 27.1	1075± 195.9	2332± 135.5	213± 45.0	2350± 69.5	608± 130.0
Dry weight of vine (gm.)	339± 22.3	99± 5.3	179± 41.5	353± 28.6	28± 5.2	380± 20.3	78± 19.6
Fruit production							
Total fresh weight (gm.) of fruit per plant	1747± 228.4	447± 84.8	760± 73.9	1925± 299.7	72± 44.2	2393± 130.7	401± 113.2
No. of fruit ripened per plant	15± 1.7	6± 1.0	11± 1.5	20± 2.8	2± 1.0	21± 1.7	4± 0.7
No. of immature fruit per plant	21± 2.9	2± 0.6	1± 1.2	22± 3.6	0± 0.0	25± 3.2	6± 3.7
Total no. of fruit per plant	36± 4.0	8± 1.2	12± 0.8	42± 4.7	2± 1.0	46± 3.5	10± 4.0
Mean weight ripe fruit (gm.)	92± 5.0	81± 7.7	68± 6.0	77± 3.9	50± 8.6	87± 8.6	84± 5.1

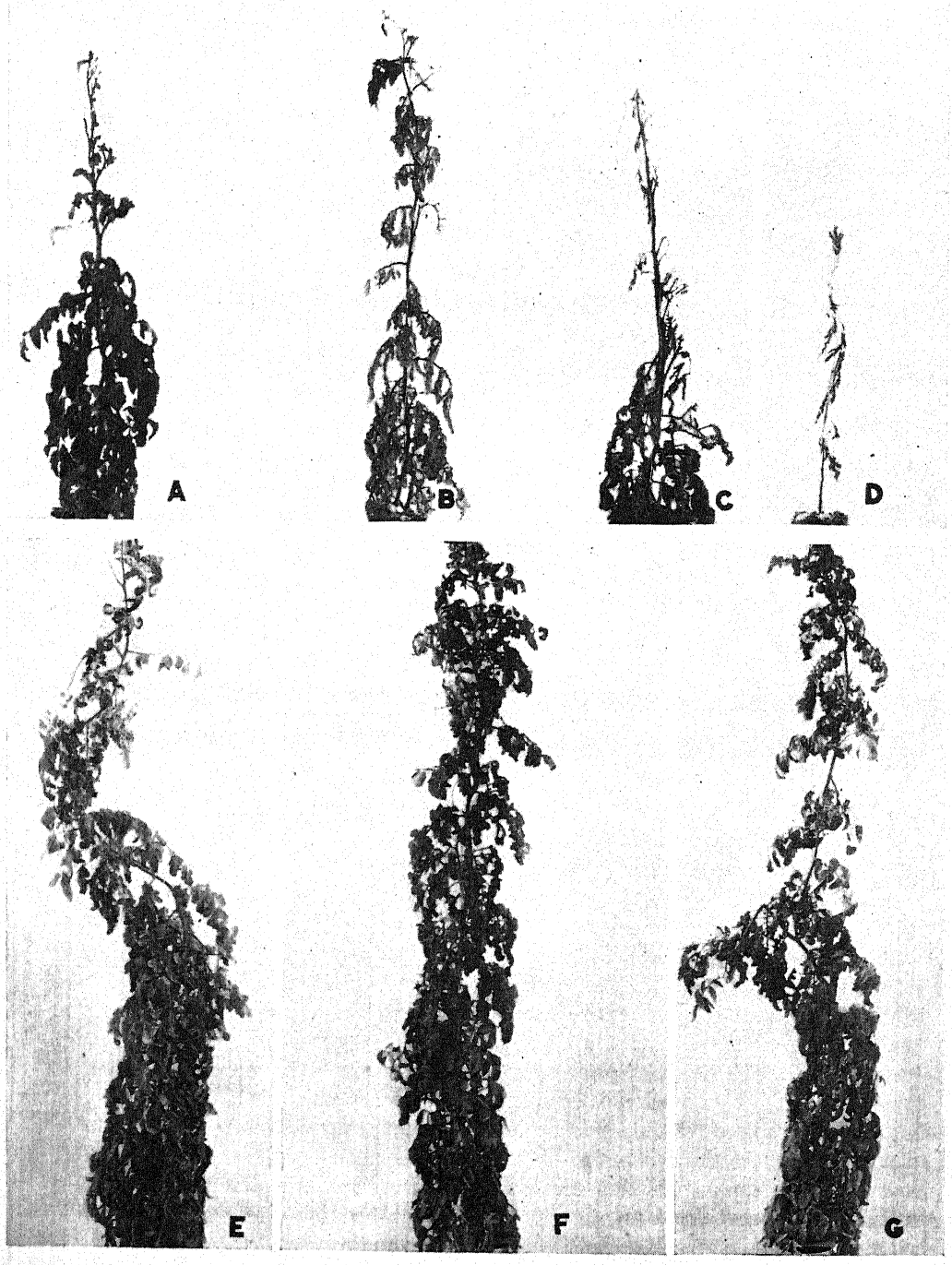


FIG. 2.—Typical tomato plants grown with various micro-nutrient deficient culture solutions together with control plant: *A*, iron-deficient; *B*, zinc-deficient; *C*, manganese-deficient; *D*, copper-deficient; and *F*, control plant. *E*, plant grown with nutrient with no added molybdenum, and *G*, with no added boron, in nutrient medium.

alyses on the second, fourth, and sixth fruits and the leaflets from deficient plants provide an index of the severity of the deficiency (table 3). When compared with other analytical data, these show that the control fruits had normal amounts of manganese (6). The lower leaflets of the control plants contained approximately 5.5 times as much manganese per unit dry weight as the leaflets near the top of the plant. Thus there is a gradient of manganese content within the plant which increases from top to bottom. The data for the manganese content of fruits are computed on a fresh-weight basis. Since most tomato fruits contain 7–8 per cent dry matter, the value given in the table may be multiplied by an approximate factor of 12 to obtain values comparable with those of the leaflets. It is evident that the concentration of manganese in fruits is less on a dry-weight basis than in leaflets from any portion of the plant. The deficient fruits contained about one-ninth as much manganese as did those produced on the control plants. The leaflets of the deficient plants contained only small amounts of manganese, and there were no

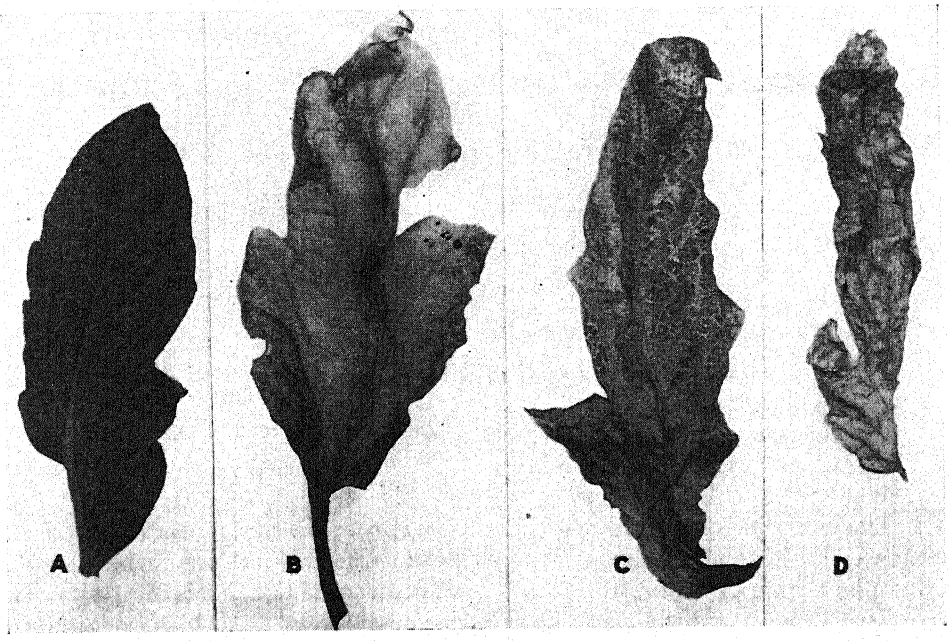


FIG. 3.—Typical leaflets from tomato plants grown with various micro-nutrient deficient culture solutions together with control: *A*, control; *B*, manganese-deficient; *C*, zinc-deficient; and *D*, copper-deficient.

TABLE 3

MANGANESE ANALYSES (P.P.M.) OF PLANTS GROWN IN COMPLETE AND MANGANESE-DEFICIENT CULTURE SOLUTIONS

Plant part	Complete		Mn deficient	
	Mean ± S.E.	No. of analyses	Mean ± S.E.	No. of analyses
Fruit*	1.7 ± 0.17	11	0.2 ± 0.02	9
Leaflets†				
Top one-third of plant	69.7 ± 9.08	7	5.6 ± 1.33‡	5
Middle one-third of plant	248.3 ± 29.95	7	5.3 ± 0.94	6
Lower one-third of plant	398.0 ± 23.79	7	5.4 ± 0.67	7

* Computed per unit fresh weight of fruit.
† Computed per unit dry weight of leaflets.
‡ Many leaflets dead at time of harvest.

significant differences in the concentration of this element in leaflets from different positions on the plant. The gradient found in controls was not evident in the deficient plants. The relative proportion of manganese in fruits as compared with leaflets was greater in the manganese-deficient plants than in the controls, indicating some accumulation of manganese in the fruit at the expense of vegetative parts when the supply of this element is limited.

Zinc deficiency

The symptoms of zinc deficiency appeared rather late in the life-span of the plant. The first evident symptom was a chlorosis between the veins of the lowermost leaflets. The veins and veinlets remained green. The chlorotic condition progressed upward on the plant, and, when the leaflets on the middle part were affected, the lowermost leaflets were a clear light brown which was not limited to the blades but included veins and veinlets as well. When the leaflets at the top of the plant were chlorotic, the middle leaflets were brown and the blades of the lowermost leaflets were speckled (fig. 3*C*). These stages followed in sequence, and in some cases the upper leaflets appeared normal in all respects while the lowermost were completely brown and speckled. Eventually, necrotic areas appeared at the margins of the leaflet and progressed toward the middle. At the conclusion of the experiment, all leaflets on a plant were a clear brown in color, speckled, and had necrotic areas (fig. 2*B*). The leaves as a whole were usually smaller than those of control plants, and there was a tendency for the leaflets to curl inward.

Vegetative growth was significantly less than that of control plants (table 2). Fruit production was also significantly less. As was the case for manganese, certain characteristic symptoms were evident on zinc-deficient fruits. They were small as compared with the controls, and the coloring of the fruit was a mottled red or a reticulum of red and yellow-orange. The mottling was particularly prevalent near the distal portion and, as the fruit matured, progressed toward the base. It was not evident on all fruits, although it was most prevalent on those from plants which exhibited the more severe deficiency symptoms.

TABLE 4

ZINC ANALYSES (P.P.M.) OF PLANTS GROWN IN COMPLETE AND ZINC-DEFICIENT CULTURE SOLUTIONS

Plant part	Complete		Zn deficient	
	Mean ± S.E.	No. of analyses	Mean ± S.E.	No. of analyses
Fruit*	1.9 ± 0.13	9	0.6 ± 0.06	8
Leaflets†				
Top one-third of plant	28.0 ± 5.50	5	23.4 ± 5.84	5
Middle one-third of plant	26.9 ± 3.43	5	14.4 ± 1.61	5
Lower one-third of plant	29.8 ± 3.36	5	16.5 ± 1.92	5

* Computed per unit fresh weight of fruit.
† Computed per unit dry weight of leaflets.

Analytical data (table 4) indicate a uniform distribution of zinc among leaflets of control plants and that the concentration found in control fruits is approximately 80 per cent of that of the leaflets. The content of zinc in the leaflets of deficient plants compared with that of the controls shows that, while no significant differences occur in the upper one-third of the plant, significantly less zinc is present in the lower two-thirds. Relatively large standard errors are involved in the analytical data of the top leaflets, and greater variability with respect to zinc content was evident between plants in both the control and the deficient treatment. Fruits produced by

zinc-deficient plants contained approximately 30 per cent as much zinc as those from controls and approximately 30 per cent of the amount in deficient leaflets.

Iron deficiency

When the seedlings were transplanted into cultures which contained no added iron, they were about 3 inches tall and extremely chlorotic. They were deficient in iron as well as in other elements. Although they became normal in appearance, they failed to make appreciable growth after transplanting, and it was apparent that some addition of iron was necessary if the plants were to produce fruits. Since the primary objective of this experiment was to produce iron-deficient fruits for vitamin analyses, and since the distribution of iron in fruit and vegetative parts of the plant was unknown, it was inadvisable to add iron directly to the nutrient medium, as fruits containing relatively large quantities of iron might result. Statements in the literature imply that iron is relatively immobile in plants (35). If true, it should be possible theoretically to spray the lowermost leaves of a plant with a stable emulsion of iron so that they would become green. If this occurred, the manufactured foods might then be conducted to fruits on the upper portions lacking the iron. Accordingly, an emulsion of ferric citrate (concentration of 1:1000) was prepared (24) and atomized on to the lower leaflets July 1, 1942. Within a period of 2 weeks, not only the lower leaflets but the unsprayed upper leaflets had become green on five of the seven plants. In two cases the application of the emulsion resulted in no change, either in upper or lower leaflets, and the plants were sprayed again on July 20. Subsequently all leaflets of these two plants also became green. The five plants which had become entirely green were maintained in their respective cultures with no further addition of iron, and ultimately—after a period of about 6 weeks—three of the plants again began to develop characteristic iron-deficiency symptoms. In two of these cases the symptoms were so severe that the young expanded leaflets at the apex were a yellowish white color. At the conclusion of the experiment the three plants were light green in color, with a definite yellowish white color at the top of the plant (fig. 2*A*). The fruits and vegetative parts contained significantly less iron than did the controls. The remaining four plants (including the two which were sprayed twice) failed to develop striking deficiency symptoms and did not contain appreciably less iron. In subsequent data, only the results of the three clearly deficient plants were included.

Vegetative growth was clearly affected by the limited supply of iron (table 2; fig. 2*A*). In all characters used as criteria of vegetative vigor, significant differences are demonstrable between the data for iron-deficient and for control plants. It is also evident that the treatment seriously affected all characters used as criteria of fruitfulness, except the size of mature fruit, for which no statistically significant differences are evident. The deficient fruits which developed were a waxy, yellowish green color and though mature remained green longer than did control fruits. The ripening process with its accompanying red color was delayed; the fruits finally became entirely pink, but a dark red color was never attained.

From table 5 it is evident that the top leaflets of control plants contained less iron than those located at the bottom of the plant, since a mean difference of 54 ± 18.4 p.p.m. (10 degrees of freedom are available, $t = 2.93$) is demonstrable.

The control fruits contained approximately 20 per cent as much iron as the leaflets on a dry-weight basis. Fruits produced by the three deficient plants contained significantly less iron than control fruits, since a mean difference of 1.9 ± 0.53 p.p.m. (9 degrees of freedom are available, $t = 3.58$) is evident with respect to iron content. The leaflets of deficient plants also contained significantly less iron than the controls, and the same distribution of iron was maintained in the plant when the supply of iron was limited.

TABLE 5

IRON ANALYSES (P.P.M.) OF PLANTS GROWN IN COMPLETE AND IRON-DEFICIENT CULTURE SOLUTIONS

PLANT PART	COMPLETE		FE DEFICIENT	
	Mean ± S.E.	No. of analyses	Mean ± S.E.	No. of analyses
Fruit*	3.3 ± 0.41	6	1.4 ± 0.33	5
Leaflets†				
Top one-third of plant	175 ± 14.9	6	74 ± 18.4	3
Middle one-third of plant	199 ± 9.0	6	128 ± 8.7	3
Lower one-third of plant	229 ± 10.8	6	167 ± 8.6	3

* Computed per unit fresh weight of fruit.
† Computed per unit dry weight of leaflets.

COPPER DEFICIENCY

With the exception of the iron-deficient plants, those deficient in copper were the first to show recognizable symptoms. A gray-green coloration of the entire plant was first discernible about June 25, when the plants were 67 days old (25 days after transplanting), and was clearly evident by June 30. This coloration was followed approximately 1 week later by distinct chlorosis of the lower leaflets. The latter condition first appeared immediately adjacent to the veins and gradually spread into the islets. The veins became dark brown and apparently filled with a brownish deposit. Microscopic examination showed no cellular deterioration at this stage. Subsequently the chlorotic lower leaflets became bronzed, extremely flexible, and the leaf petioles formed unusual angles with the stems. The bronzed condition was followed by complete browning of the leaflets, with necroses at the margins and black veins (fig. 3*D*). Lastly a clear brown color with an accompanying brittle quality of the leaves indicated death. Brown woody spots developed on the surface of the stems and petioles. The stems were thin and spindly, while all leaves and leaflets were relatively small. By September 2, when the plants were 135 days old, five of the seven replications had only three or four green leaves at the apex of the plant. At the conclusion of the experiment, when the plants were 165 days old, most of the stem and all leaves were dead on each plant (fig. 2*D*).

From table 2 it is clear that a lack of copper resulted in the smallest plants in the experiment. Highly significant differences are demonstrable between the data for copper-deficient and the control treatments in all characters used as criteria of vegetative vigor. Similarly, the data for fruitfulness show highly significant differences for all characters. Each plant flowered and set two fruits early in the experiment, and all subsequent flowers abscised at the pedicel joint. The mature fruits were small (table 2), but the ripening process with respect to colors which developed and rate of maturation appeared comparable with that of the control fruits.

The copper content showed no consistent relationship to symptoms which developed on the deficient plants. Furthermore, the variability of the results could not be explained or correlated with

the growth data, hence the copper analyses are not reported. It is planned to obtain further analytical results from copper-deficient and normal tomato plants in subsequent experimentation.

Molybdenum and boron

The data for the growth of plants grown with nutrient solutions to which no molybdenum was added (table 2) agree within the limits of error with similar data for control plants, and in no case is a significant difference demonstrable. There was no evident external vegetative symptom which could be correlated with a limited supply of this element (fig. 2*E*). The data for the number of ripe and developing fruits, the total number of fruits, and the total fresh weight of fruit produced agree closely with similar data for the control plants; but there are indications that the size of ripe fruits produced by molybdenum-deficient plants was smaller than that of control fruits. A mean difference of 15 ± 6.3 gm. (33 degrees of freedom are available, $t = 2.38$) is shown in the data, and while this difference is not significant according to the arbitrary standards required in this experiment, fruit size was the only external character examined which showed indication of less growth as a result of limited molybdenum supply.

The results of molybdenum analyses are given in table 6. It is evident that lower leaflets of control plants contained significantly more molybdenum than top leaves, since a significant mean difference of 2.29 ± 0.745 p.p.m. is demonstrable in the data (12 degrees of freedom are available, $t = 3.04$). The concentration of molybdenum in the fruits on a dry-weight basis was approximately 30 per cent of that of leaflets. The fruits contained slightly less than 0.01 ± 0.001 p.p.m. of molybdenum, and its concentration in the leaflets of the treated plant was approximately 10 per cent of that of the controls. In these plants there does not seem to be any uniform trend for molybdenum content of the leaflets, although there is an indication that lower leaflets contain more than those of the middle portion of the plant. The mean difference of 0.18 ± 0.068 p.p.m. ($t = 2.65$) is not significant.

TABLE 6

Molybdenum analyses (p.p.m.) of plants grown in complete and molybdenum-deficient culture solutions

Plant part	Complete		Limited Mo supply	
	Mean ± S.E.	No. of analyses	Mean ± S.E.	No. of analyses
Fruit*	0.06 ± 0.005	6	0.01 ± 0.001	11
Leaflets†				
Top one-third of plant	2.53 ± 0.554	7	0.26 ± 0.059	7
Middle one-third of plant	3.48 ± 0.365	7	0.21 ± 0.034	7
Lower one-third of plant	4.82 ± 0.512	7	0.39 ± 0.059	7

* Computed per unit fresh weight of fruit.
† Computed per unit dry weight of leaflets.

Boron was included in the nutrient solution supplied to the control plants to preclude possibility of a boron deficiency. The treatment receiving no boron was included largely as a test of the boron-supplying capacity of Pyrex glass. No significant differences with respect to any character used as a criterion of growth and fruitfulness were obtained between the control plants (receiving added boron) and those receiving nutrient to which no boron was added (table 2; fig. 2*G*). Boron analyses of leaflets from plants of both of these treatments were made when the plants were 54 days old (14 days after transplanting) and again when 135 days old. At the earlier date the leaflets of plants in the first treatment contained 62 p.p.m. of boron, while leaflets from the

latter treatment contained 39 p.p.m. of boron. When the plants were 135 days old the boron content of leaflets taken from plants of both treatments was the same. Thus in this experiment the Pyrex-glass gravel supplied adequate boron for normal growth and reproduction.

VITAMIN CONTENT OF FRUITS

The first, third, fifth, and seventh fruits which ripened on each plant were assayed for ascorbic acid, riboflavin, and provitamin A. No attempt was made to select fruits from any particular cluster, and the usual situation with respect to the control plants was that the first and third fruits ripened on the first fruit cluster, the fifth fruit was harvested from the second fruit cluster, and the seventh fruit ripened on the third fruit cluster. One additional point is pertinent to any interpretation of the results. The first fruits obtained from each of the seven replications in any one treatment ripened within 6 days of one another, and a similar situation was found for the third fruits. The fifth fruits ripened within 10 days and the seventh fruits within 2 weeks of one another. While the extremes with respect to ripening date overlap, there are clearly different mean dates for the time at which the first, third, fifth, and seventh fruits ripened. Furthermore, the different treatments ripened their first fruits at approximately the same time (within a week of one another), so that the first fruit from any replication of any deficient treatment ripened under environmental conditions similar to those of the first fruit from any replication of the control treatment. Any differences within or between treatments with respect to the ripening date of any given fruit appear to be distributed at random in the data.

Since the results previously discussed have characterized the deficiency conditions produced in this experiment, and since mineral analyses were made on both fruits and leaflets of each plant, it was possible in most cases to determine which plants included in any treatment were clearly deficient in any given mineral element and whether or not they produced fruit deficient in a given element. It is evident from the data that four plants of the treatment where the supply of iron was limited did not develop iron-deficiency symptoms, and the results obtained from iron analyses of the fruits from these plants were comparable with those of controls. The vitamin data on these fruits were discarded. Using similar criteria, the results from two plants from the treatment which had no zinc added to the nutrient medium were not used. The results of vitamin analyses on fruits from all other plants were considered valid for purposes of this experiment.

Significant differences between treatments are inherent in the data for ascorbic acid and riboflavin, while none were demonstrable for provitamin A (table 1). Riboflavin was the only vitamin in this experiment which was significantly affected by position of the plants in the greenhouse. One other point illustrated in the analysis of variance is also pertinent to any interpretation of the results. The data for each vitamin were tested for the possible presence of significant differences between values for the first, third, fifth, and seventh fruits. This is in effect a further test of the influence of environment, since such a classification is chronological and the last fruit to ripen on a plant matures under environmental conditions different from those of the first fruit. In this respect, significant differ-

ences between fruit sequences were found for riboflavin only. Thus the riboflavin content of tomato fruits seems to be greatly affected by environmental conditions, since (*a*) significant differences with respect to riboflavin content were correlated with the time at which the fruits were harvested and (*b*) riboflavin content was also correlated with the position of the plant in the greenhouse. This in no way invalidates inter-treatment comparisons, since the effects of the position of the plant and the time at which a fruit ripened were measured and removed from inter-treatment comparisons in the analysis of variance.

TABLE 7

VITAMIN ANALYSES OF TOMATOES GROWN WITH MICRO-NUTRIENT DEFICIENCIES

Treatment	Ascorbic acid*		Riboflavin†		Provitamin A‡	
	Mean±S.E.	No. of analyses	Mean±S.E.	No. of analyses	Mean±S.E.	No. of analyses
Complete nutrient	20.0±0.46	26	0.30±0.010	23	4.0±0.22	24
Mn deficiency	20.2±0.49	18	0.33±0.010	19	4.1±0.20	17
Zn deficiency	21.5±0.70	17	0.26±0.017	18	4.4±0.37	14
Cu deficiency	20.5±1.05	6	0.33±0.013	5	5.1±0.38	6
Mo supply limited	19.6±0.39	27	0.31±0.013	26	4.0±0.19	26
Fe deficiency	26.1±0.55	6	0.34±0.018	5	3.4±0.30	6
B not added to nutrient	19.6±0.54	26	0.30±0.008	26	4.1±0.28	26

* Milligrams per 100 gm. fresh weight fruit.
† Micrograms per gm. fresh weight fruit. ‡ Micrograms per gm. fresh weight fruit.

The results of the vitamin analyses are given in table 7. Individual errors which measure variation between plants and between fruits within plants were computed for each treatment in these data. Significantly more ascorbic acid was found in fruits when the supply of iron was limited than in control fruits, since a significant mean difference of 6.1±0.72 mg. per 100 gm. is shown in the data (30 degrees of freedom are available, $t=8.47$). The ascorbic-acid values for fruits produced in other treatments did not differ from those of the control treatment within the limits of error in the experiment. When data for the deficient treatments are compared individually with those of the control treatment, no significant difference in the riboflavin content of the fruits is demonstrable. It is evident that the individual errors are high, owing to the fact that they include variability obtained as a result of positional effects in the greenhouse and the effects of sequence number. It is possible, by using a generalized error from the analysis of variance (where some sources of variability other than those inherent within treatments are measured and removed), to demonstrate significant differences between treatments. Regardless of the presence or lack of significance, however, the differences obtained are small and probably of no practical value.

The data obtained with respect to provitamin A indicate that the treatments did not influence the content of this vitamin in the fruits. The variability is high, and the analysis of variance would indicate that it occurs at random, since in this experiment no significant effects of position in the greenhouse or of

the time of fruit ripening are demonstrable in the data. The values reported for provitamin-A content of these fruits are in agreement with those of previous experiments (15).

Discussion

The evidence necessary to establish the essentiality of mineral elements for the growth of higher plants has been adequately discussed (3, 26). A quantitative approach as a method of physiological research is necessary in order to establish the essential nature of many elements which may be required in amounts smaller than the incidental impurities which can be removed from culture solutions by present techniques. It is also essential to establish the degree or the severity of the deficiencies, which may be correlated with other variables. It seems obvious that the terms somewhat deficient, deficient, or very deficient are relative in meaning. Their use and interpretation, however, can be more exact if they are defined in terms of chemical analysis, which provides a scale of reference. This procedure in no way invalidates or discards the use of vegetative symptoms as a criterion.

In this experiment the development of external deficiency symptoms on vegetative parts of the plant can be closely correlated with data obtained by chemical analyses. Those plants developing symptoms which could be correlated with a limited supply of an element were found upon analysis to contain significantly smaller amounts of that element than control plants. Conversely, plants which failed to develop deficiency symptoms were found to contain quantities of a given element comparable with that found in controls. The treatment involving no addition of molybdenum to the nutrient medium would be an exception to the latter statement. Within a treatment, the replications differed in the severity of the symptoms which they developed, and plants which developed the most severe symptoms invariably contained the smallest quantities of the element under consideration.

Since ARNON and STOUT (4) have described external molybdenum-deficiency symptoms on the tomato plant, we have avoided designating those plants which were grown with a limited molybdenum supply as deficient. In this experiment no external deficiency symptoms were recognizable, but chemical analyses have shown that they contain approximately one-tenth of the quantity of molybdenum present in control plants. As far as we are aware, no published data are available concerning the molybdenum content of plants with clearly developed external deficiency symptoms. Such data would be helpful in an interpretation of these results. It is possible that contaminants present in these cultures supplied enough molybdenum to support growth and fruitfulness comparable with that obtained in the complete nutrient. If so, certainly only very minute quantities of molybdenum are necessary for normal growth of the tomato plant.

Mineral analyses on leaflets from different parts of the plant have indicated correlations involving the distribution of some micro-nutrients within the plant. There was a suggestion that the first external symptom of a deficiency condition occurred in that part of the plant where the content of a micro-nutrient was small as compared with other parts. Also, at any given time, the severity of the external symptoms seemed to be greatest in that part of the plant. For instance, lower leaflets of all control plants contained

significantly more iron and manganese than did leaflets at the top of the plant. In iron- and manganese-deficient plants the first symptoms of the deficiency appeared at the top of the plant and subsequently became more severe at that point.

Recently the concept that manganese is concerned in the elaboration of ascorbic acid has received some support. This concept was first developed from biochemical studies of liver tissue (41, 42), although recent experiments (9) have failed to confirm this conclusion. HESTER (21–23) has stated that the addition of manganese to soils deficient in this element resulted in increased content of ascorbic acid in tomato fruits. The results reported here have shown that the ascorbic-acid content of manganese-deficient tomato fruits is not different from that of control plants. The deficient condition produced in this experiment would not be tolerated in field-crop production, owing to the limited growth and fruitfulness. This seems to indicate that manganese is not directly concerned in ascorbic-acid synthesis but in no way eliminates the possibility that additions of manganese to soils may alter their physical and chemical properties. In such instances the effect of manganese would thus be indirect. This is discussed elsewhere (29).

In this experiment a relatively large difference in the content of manganese, zinc, iron, molybdenum, and probably copper in the plant was experimentally induced. The micro-nutrient content of these plants is in some cases lower than any values previously reported. It may be deduced that a limited supply of zinc, copper, manganese, and molybdenum showed no correlation with the vitamin content of tomato fruits under these conditions. The results indicate the necessity of examining with care data in which significant correlations exist, since other conditions in experimental technique may be of primary importance. In this respect it should be evident that interpretation of these results depends on the following interpretations of a deficient condition. If the deficient condition results from lack of an adequate quantity of an element necessary for some phase of cellular metabolism, an understanding of the role of various micro-nutrients is desirable. On the other hand, SHIVE (44) has suggested that symptoms resulting from a limited supply of one element may be caused by toxic effects of another. The validity of this interpretation can be tested only by investigations which consider toxicity effects. If the viewpoint is accepted that the "balance" of micro-nutrient concentrations is the controlling or causal factor, then investigations relative to the effects of varied proportions of micro-nutrients are indispensable. In any event it is desirable to measure quantities of micro-nutrients within the plant as well as in the substrate, to clarify the "lack," "toxicity," "balance," or other relationship.

Summary

1. An experiment was designed to test the effects of manganese, copper, zinc, molybdenum, and iron deficiencies on the ascorbic-acid, riboflavin, and provitamin-A content of tomato fruits. The criteria of a micro-nutrient deficiency included measurements of growth and fruitfulness, development of deficiency symptoms, and chemical analyses of both leaflets and fruits. All data were reduced and analyzed by statistical methods.

2. An apparatus made from Pyrex glass is described for use in micro-nutri-

ent studies in the greenhouse when it is desired to grow plants to maturity and to test the effects of a number of treatments in replicated cultures.

3. External deficiency symptoms of vegetative parts are described for plants deficient in manganese, copper, zinc, and iron. A characteristic manganese-deficiency symptom which occurs in tomato fruits is described. A possible deficiency symptom shown by fruits with respect to zinc is also described.

4. Deficiencies of manganese, copper, zinc, and iron resulted in considerably less growth and fruitfulness than was measurable in control plants. The growth and fruitfulness of plants grown with a limited supply of molybdenum are comparable with those of control plants.

5. In general, chemical analyses of leaflets and fruits from plants grown with limited supplies of manganese, iron, zinc, and molybdenum showed significantly smaller quantities of the respective element than did the control plants. Development of the symptoms associated with a particular deficiency with respect to their position on the plant was closely correlated with the distribution of the element in the plant. The latter was determined by chemical analyses of leaflets from the top, middle, and lower thirds of the plant.

6. The ascorbic-acid content of fruits was not affected by limiting the supply of manganese, zinc, copper, and molybdenum, although growth and fruitfulness were seriously affected by a lack of manganese, zinc, and copper. Fruits from iron-deficient cultures contained 30 per cent more ascorbic acid than did control fruits on a fresh-weight basis. The quantity of fruit produced by iron-deficient plants, however, was significantly less than that of control plants.

7. Slight variations in riboflavin content of fruits could be correlated with the effect of micro-nutrient deficiencies. The magnitude of these variations was small and of no practical significance. The data show a pronounced effect on riboflavin content of variations in climatic environmental conditions such as occur with different positions in the greenhouse and with a different date of fruit maturation.

8. Provitamin-A content of the fruits was not significantly affected by a limited supply of the micro-nutrients. No significant effect of environmental factors was obtained with respect to this vitamin.

9. A discussion of the possible interpretation of these results is included.

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LITERATURE CITED

1. Arnon, D. I., Ammonium and nitrate nutrition of barley at different seasons in relation to hydrogen-ion concentration, manganese, copper, and oxygen supply. Soil Sci. 44:91–114. 1937.
2. ———, Microelements in culture-solution experiments with higher plants. Amer. Jour. Bot. 25:322–325. 1938.
3. Arnon, D. I., and Stout, P. R., The essentiality of certain elements in minute quantity for plants, with special reference to copper. Plant Physiol. 14:371–375. 1939.
4. ———, and ———, Molybdenum as an essential element for higher plants. Plant Physiol. 14:599–602. 1939.
5. Association of Official Agricultural Chemists. Methods of analysis. 5th ed. Pp. 128. 1940.
6. Beeson, K. C., The mineral composition of crops with particular reference to the soils in which they were grown. U.S.D.A. Misc. Publ. 369. 1941.
7. ———, The minor elements in agriculture. Tabulae Biologicae. 18:61–65. 1940.
8. Bernstein, L., Hamner, K. C., and Parks,

R. Q., Influence of mineral nutrition, soil fertility, and climate on carotene and ascorbic-acid content of turnip greens. Unpublished.
9. BOYER, P. D., SHAW, J. H., and PHILLIPS, P. H., Studies on manganese deficiency in the rat. Jour. Biol. Chem. 143:417–425. 1942.
10. COULSON, E. G., Report on (the determination of) copper (in foods). Jour. Assoc. Off. Agr. Chem. 20:178–191. 1937.
11. COWART, F. F., The composition of quality fruit and the effect of certain fertilizer practices on the composition and time of maturity of oranges and grapefruit. Citrus Grower (Florida) 3. No. 35. 1941.
12. COWLING, H., and MILLER, E. J., Determination of small amounts of Zn in plant materials. A photometric dithizone method. Ind. Eng. Chem. Anal. Ed. 13:145–149. 1941.
13. DRABKIN, D. L., Report on (the determination of) copper (in foods). Jour. Assoc. Off. Agr. Chem. 22:320–333. 1939.
14. EATON, F. M., Use of nitric acid in control of pH and nitrate levels in nutrient solution. Plant Physiol. 16:834–836. 1941.
15. ELLIS, G. H., and HAMNER, K. C., The carotene content of tomatoes as influenced by various factors. Jour. Nutrition 25:1943.
16. ELLIS, G. H., BERNSTEIN, L., and THOMPSON, J., Methods for the determination of provitamin A in certain crops. Unpublished.
17. FISHER, R. A., The design of experiments. Oliver and Boyd, London. 1937.
18. HAMDALLAH, ABU EL WAFA, Vitamin C content of plants grown without iron and magnesium. Protoplasma 32:31–43. 1939.
19. HAMNER, K. C., LYON, C. B., and HAMNER, C. L., Effect of mineral nutrition on the ascorbic-acid content of the tomato. BOT. GAZ. 103:586–616. 1942.
20. HAMNER, K. C., ELLIS, G. H., PARKS, R. Q., and MACHATA, R., Thiamin, nicotinic acid, and riboflavin content of turnip greens as influenced by mineral nutrition, soil fertility, and climate. Unpublished.
21. HESTER, J. B., Manganese and vitamin C. Science 93:401. 1941.
22. HESTER, J. B., and HALL, H. F., Quality tomatoes through sound farming practices. Agr. Res. Bull. no. 4 of Campbell Soup Co. 1942.
23. ———, and ———, Influence of soil fertility upon the quality of tomatoes. Soil Sci. Soc. Amer. Proc. 6:243–245. 1941.
24. HILDRETH, A. C., and MITCHELL, J. W., Spraying is a new method of applying root-promoting substances. Florist's Review. May, 1939.
25. HILLEBRAND, W. F., and LUNDELL, G. E. F., Applied inorganic analysis. Wiley and Sons, New York. Pp. 106–109. 1929.
26. HOAGLAND, D. R., Some aspects of the salt nutrition of higher plants. Bot. Rev. 3:307–334. 1937.
27. LIPMAN, C. B., and MACKINNEY, G., Proof of the essential nature of copper for higher green plants. Plant Physiol. 6:593–599. 1931.
28. LUNDELL, G. E. F., and HOFFMAN, J. I., Outlines of methods of chemical analysis. Wiley and Sons, New York. Pp. 88–89. 1938.
29. LYON, C. B., and BEESON, K. C., Manganese and ascorbic-acid formation. Jour. Amer. Soc. Agron. 35:166–167. 1943.
30. MARMOY, F. B., The determination of Mo in plant materials. Jour. Soc. Chem. 58:275–276. 1939.
31. MAZÉ, P., Influences respectives des éléments de la solution minérale sur le développement du maïs. Ann. Inst. Pasteur 28:21–68. 1914.
32. ———, Détermination des éléments minéraux rares nécessaires au développement du maïs. Compt. Rend. Acad. Sci. Paris 160:211–214. 1915.
33. ———, Recherche d'une solution purement minérale capable d'assurer l'évolution complète du maïs cultivé à l'abri des microbes. Ann. Inst. Pasteur 33:139–173. 1919.
34. MCHARGUE, J. S., Effect of different concentrations of manganese sulphate on the growth of plants in acid and neutral soils and the necessity for manganese as a plant nutrient. Jour. Agr. Res. 24:781–794. 1923.
35. MILLER, E. C., Plant physiology. McGraw-Hill, New York. Pp. 334–335. 1938.
36. MORELL, S. A., Rapid photometric determination of ascorbic acid in plant materials. Ind. Eng. Chem. Anal. Ed. 13:793–794. 1941.
37. NAFTEL, J. A., Colorimetric micro-determination of B. Ind. Eng. Chem. Anal. Ed. 11:407–409. 1939.
38. POWERS, W. L., Boron in relation to soil fertility in the Pacific northwest. Soil Sci. Soc. Amer. Proc. 4:290–296. 1939.
39. ROGERS, L. H., Comparison of micro methods for the determination of Mo in plants and soils. Doctor's Dissertation, Cornell University. 1941.
40. ROY, W. R., and BAHRT, G. M., The effect of zinc, iron, manganese and magnesium, applied to frenched and bronzed orange groves, on the vitamin C content of oranges. Proc. Florida State Hort. Soc. 53:34–38. 1940.
41. RUDRA, M. N., Role of manganese in the biochemical synthesis of ascorbic acid. The precursors of ascorbic acid in rat tissue. Osterr. Chem. Zeittg. 42:315–317. 1939.
42. ———, Role of manganese in the biological synthesis of ascorbic acid. Nature 144:868. 1939.
43. SAYWELL, L. G., and CUNNINGHAM, B. B., Determination of iron. Colorimetric o-phenanthroline method. Ind. Eng. Chem. Anal. Ed. 9:67–69. 1937.
44. SHIVE, J. W., Significant roles of trace elements in the nutrition of plants. Plant Physiol. 16:435–445. 1941.

45. SNEDECOR, G. W., Statistical methods. Collegiate Press, Ames, Iowa. 1938.
46. SNELL, E. E., and STRONG, F. M., A microbiological assay for riboflavin. Ind. Eng. Chem. Anal. Ed. 11:346–350. 1939.
47. SOMMER, A. L., Further evidence of the essential nature of zinc for the growth of the higher green plants. Plant Physiol. 3:217–221. 1928.
48. ———, Copper as an essential for plant growth. Plant Physiol. 6:339–345. 1931.
49. SOMMER, A. L., and LIPMAN, C. B., Evidence of the indispensable nature of zinc and boron for higher green plants. Plant Physiol. 1:231–249. 1926.
50. STEINBERG, R. A., Nutrient-solution purification for removal of heavy metals in deficiency investigations with *Aspergillus niger*. Jour. Agr. Res. 51:413–424. 1935.
51. STOUT, P. R., and ARNON, D. I., Experimental methods for the study of the role of copper, manganese, and zinc in the nutrition of higher plants. Amer. Jour. Bot. 26:144–149. 1939.
52. TIPPETT, L. H. C., Tracts for computers. Random sampling numbers. Cambridge University Press, London. 1927.
53. WARINGTON, K., The effects of boric acid and borax on the broad bean and certain other plants. Ann. Bot. 37:629–672. 1923.
54. YATES, F., The analysis of replicated experiments when the field results are incomplete. Empire Jour. Exp. Agr. 1:129. 1933.

EFFECTS OF SOLVENT UPON ABSORPTION SPECTRA OF CHLOROPHYLLS *A* AND *B;* THEIR ULTRAVIOLET ABSORPTION SPECTRA IN ETHER SOLUTION[1]

D. G. HARRIS AND F. P. ZSCHEILE[2]

Introduction

Spectroscopic observations on preparations of chlorophyll components have been employed most often as a test for purity. In the case of extracts from natural sources, the spectra of the components are often used as analytical standards. In both cases the choice of solvent is usually made because of desirable physical or chemical properties or because of adaptability of the solvent to extraction of the particular plant material studied.

It appears desirable at this time, when more accurate applications of spectroscopic methods are being made to the analysis of plant material for chlorophyll (15, 5, 4), to have more complete knowledge of the spectra of the chlorophyll components in different solvents. Such information will facilitate a choice of solvents that is advantageous from the spectroscopic viewpoint.

The physical condition of chlorophyll in the living green leaf has been studied from numerous points of view (10, 16, 20). Many theories make use of the positions of the absorption maxima in the spectrum of the leaf in comparison with those for known preparations or solutions. The work outlined in this paper should be of some usefulness to those who would interpret the relation of spectrum to physical condition within the plant.

Experimentation

Purification of pigments

The method employed for the isolation of chlorophyll components *a* and *b* was essentially that described by Zscheile and Comar (29), with a few slight modifications. Magnesium carbonate was employed instead of calcium carbonate to neutralize plant acids in the extraction mixture. This minimizes the possibility of substitution of calcium for magnesium in the chlorophyll molecule (19).

For the series of preparations made during this study, the sources of chlorophyll were market spinach purchased at various seasons throughout the year and young corn seedlings grown in the greenhouse. It was found that the relative concentrations of carotenes to carotenols[3] varied among the individual lots of spinach, and the procedure for removing them from chlorophyll was varied accordingly. When the precipitated chlorophyll (29) is shown by the phase test to contain carotenoids, the following is added to the earlier procedure: The crude precipitated chlorophyll, after removal of the carotenes by washing with petroleum ether, is added (with accompanying sucrose) to a mixture of 25 ml. of petroleum ether and 15–25 ml. of ethyl ether. After complete solution of the chlorophyll, the volume is made to 1 liter with petroleum ether. Three washings with distilled water remove the sucrose. The carotenols still present are completely removed by washing twice with 200 ml. of 80 per cent, thrice with 85 per cent, and twice with 90 per cent aqueous methanol. The chlorophylls are then precipitated a second time and washed with petroleum ether. No carotenoids should now be

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[3] The use of this nomenclature was explained recently (27).

present in the crude chlorophyll, and the chromatographic separation of components *a* from *b* follows the earlier method (29).

Preparations of the components by use of these modifications have yielded ether solutions with spectroscopic properties essentially identical with those determined by ZSCHEILE and COMAR.

PREPARATION OF SOLUTIONS AND SPECTROSCOPIC STUDY

After the mechanical removal of the portion of the sucrose adsorption column containing the chlorophyll component to be studied, the pigment was eluted by various solvents. Spectroscopic observations were made as soon as possible. The chlorophyll was never dried in this procedure (29); the method of elution from the column thus resembles that employed by EGLE (6) for a study of Kundt's rule. The traces of ether and petroleum ether remaining on the column, which was almost dry, are regarded as insufficient to affect the results. In the case of chlorophyll *b* in cyclohexane, the addition of a small amount of ethyl ether was necessary for elution, after which the solution was washed with water.

Acetone and methanol were purified as previously described (25). Ethyl ether was distilled after standing over solid sodium hydroxide and was free of peroxide by the vanadic-acid test of JORISSEN (2). Methyl oleate was prepared from oleic acid and methanol according to the method of ARMSTRONG, ALLEN, and MOORE (1). The yellow color was removed from this ester by vacuum distillation. Commercial olive oil was filtered through a column of sodium aluminum silicate adsorbent (11) to remove colored impurities. The remaining solvents were purified according to methods described by WEISSBERGER and PROSKAUER (23).

The solvents were divided into two groups so that the data for six solvents could be obtained within 30 hours after the preparation had been completed. The solutions in which more rapid decomposition was expected were studied first. For the first group of solvents the order was methanol, acetone, benzene, cyclohexane, carbon tetrachloride, and isopropyl ether; for the second group it was methyl oleate, olive oil, butanol-1, 2-methyl propanol-1, 2-ethyl hexanol-1, and 1, 4-dioxane. These solvents were chosen for various reasons. Since many disagreements exist in the literature on the positions of absorption bands, certain solvents were chosen to provide further tests of Kundt's rule. Other factors, such as viscosity, dipole moment, dielectric constant, and boiling point, were considered. Some solvents were used because of analytical importance; certain others because of their possible similarity to certain lipoid materials of the plastid.

Results in the visible region were obtained by the same spectroscopic instrument and methods previously described (29). The temperature of the solution was not controlled but occasional measurements during the progress of the work indicated a narrow range of 24°–26° C. Since 1-cm. absorption cells were used throughout, the concentrations, though not known accurately, were of comparable magnitude. Thus, results should be comparable with those obtained by EGLE (6) so far as uniformity of concentration is concerned.

For the ultraviolet measurements below 3260 A, a quartz hydrogen arc of the MUNCH type (17) was employed as a source of ultraviolet radiation. An incandescent source was used above 3260

A. Values were plotted at intervals of 20 A. Spectral regions isolated (9) and slit widths were as follows:

Wavelength	Slit width	Spectral region isolated
2660–3260 A	0.17 mm.	6–11 A
3260–3360	0.20	13–15
3360–3500	0.10	7–8
3500–4100	0.06	5–9

Results

Absorption spectra in different solvents

Many difficulties would have been encountered had an attempt been made to present the data as specific absorption coefficients. To determine the numerical values of such coefficients, the concentration of the solution must be known. The weight of the dissolved chlorophyll would have been difficult to obtain in many cases, owing to the difficulty of quantitative evaporation of the solvent. With some solvents, quantitative transfer to ether and subsequent spectroscopic analysis (5) would be possible, but since the main objective of this study was to determine the positions and relative heights of the absorption bands in relation to various properties of the solvents, the values of relative absorption were plotted against wavelengths. Absorption = log I_o/I, as defined by Hogness and Potter (8), where I_o and I are the intensities of radiation transmitted by the solvent-filled and solution-filled cells, respectively.

The absorption curves for chlorophyll *a* are presented in figures 1 and 3. All the observed absorption values were multiplied by appropriate factors, chosen so that the curves would all pass through one point on the graph. The wavelength of this point, 5050 A, with relative absorption value of 0.165 (absorption value chosen arbitrarily for convenience only), was chosen because it has been shown that this region of the curve is very sensitive to degradation products in the case of ethyl ether solution and because the curves obtained by Zscheile and Comar (29) for ethyl ether solutions of the chlorophyll components were plotted on the same basis for comparison. The major red maxima are plotted slightly higher than those previously published because of the slightly higher ratio values obtained on these recent preparations. This makes the curves more representative of the latest preparations. Whether the cause of this difference lies in the red or in the green, or in both, is not known. The values on the chlorophyll *a* curve for ether (29) were multiplied by a factor such that the same absorption value would result at 5050 A. The absorption was quite low at this wavelength for all the solvents studied.

Owing to the smaller quantities of component *b* available for study, only five solvents were compared. Curves presented in figure 4 were obtained by multiplying by suitable factors to provide a common point with the ether curve at 5200 A. For comparison, the ether curve (29) has been plotted also. To maintain the same relative heights of the curves for chlorophylls *a* and *b*, the chlorophyll *b* curve was also multiplied by the same factor as used for component *a* to give an absorption value of 0.263 at 5200 A, where degradation products of chlorophyll *b* are easily detected in ether solutions.

The positions of the two principal maxima in the red and blue regions of the spectrum of chlorophyll *a* in each of the thirteen solvents are presented in table 1, with the solvents arranged according to index of refraction. The R_a value has been useful to indicate decomposition in ether solutions (29). A corresponding value for each solvent has been calculated.

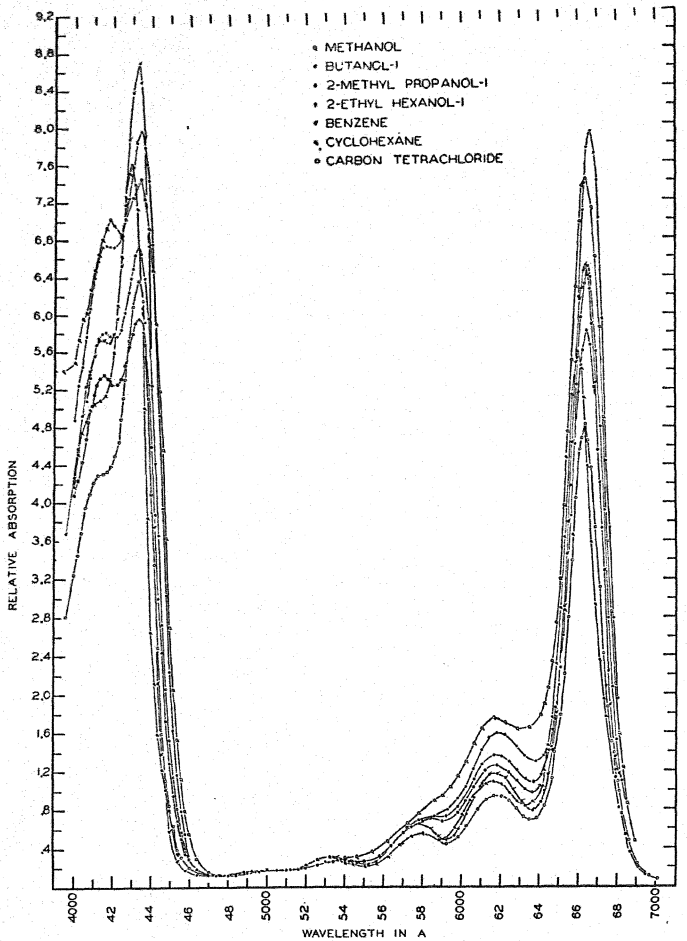


FIG. 1.—Absorption spectra of chlorophyll *a* solutions

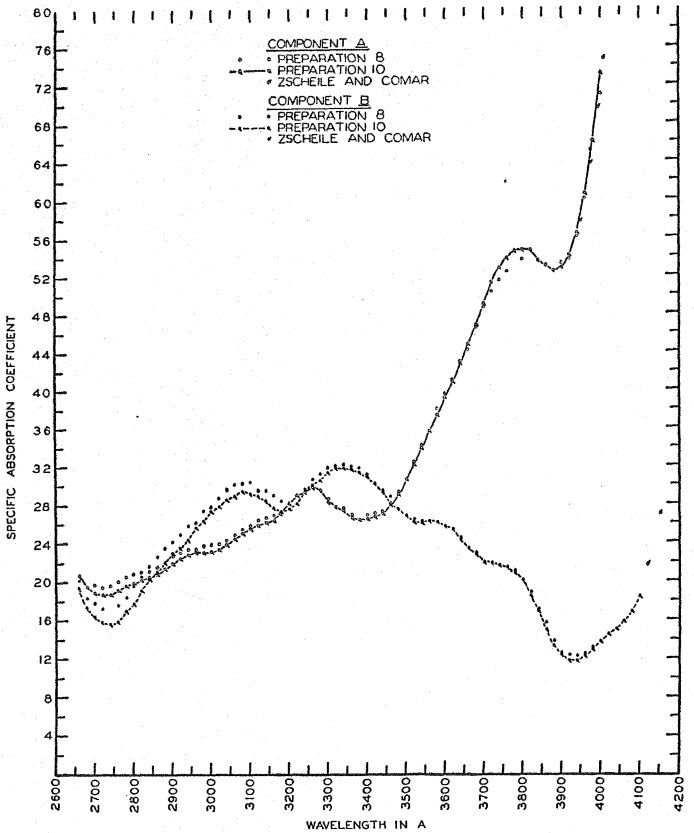


FIG. 2.—Ultraviolet absorption spectra of chlorophylls *a* and *b* in ethyl ether solution.

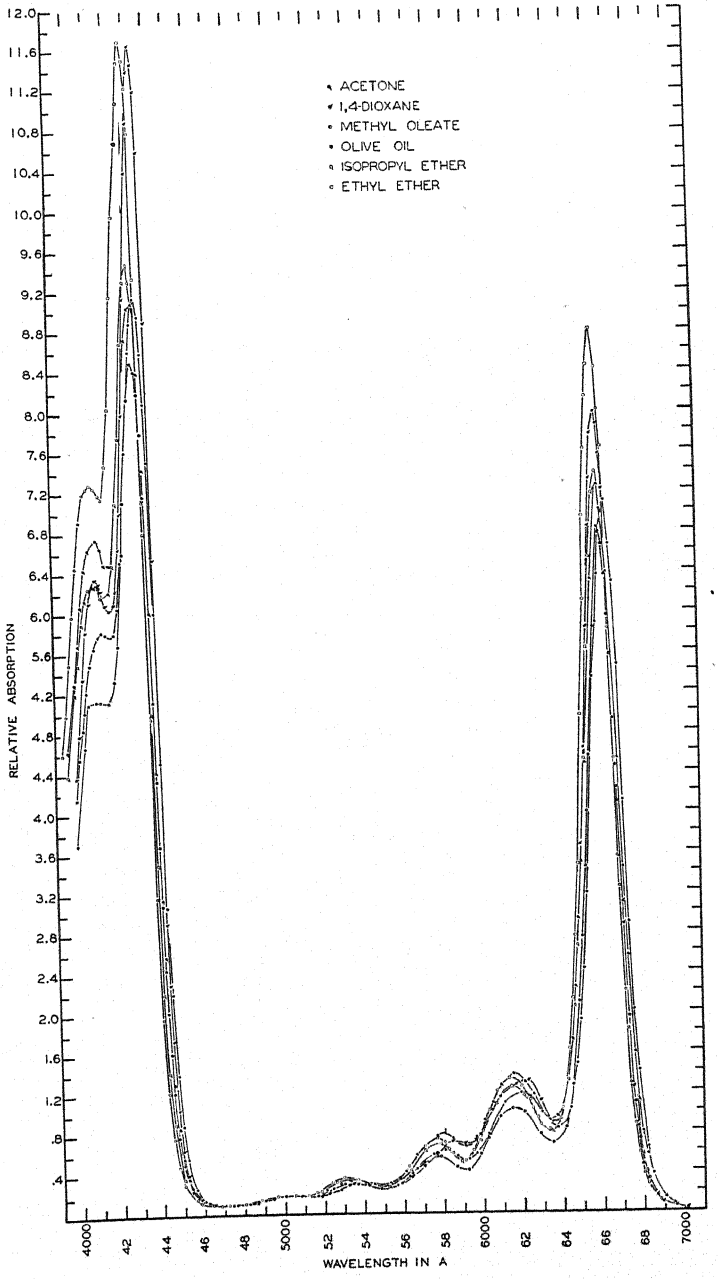


FIG. 3.—Absorption spectra of chlorophyll *a* solutions

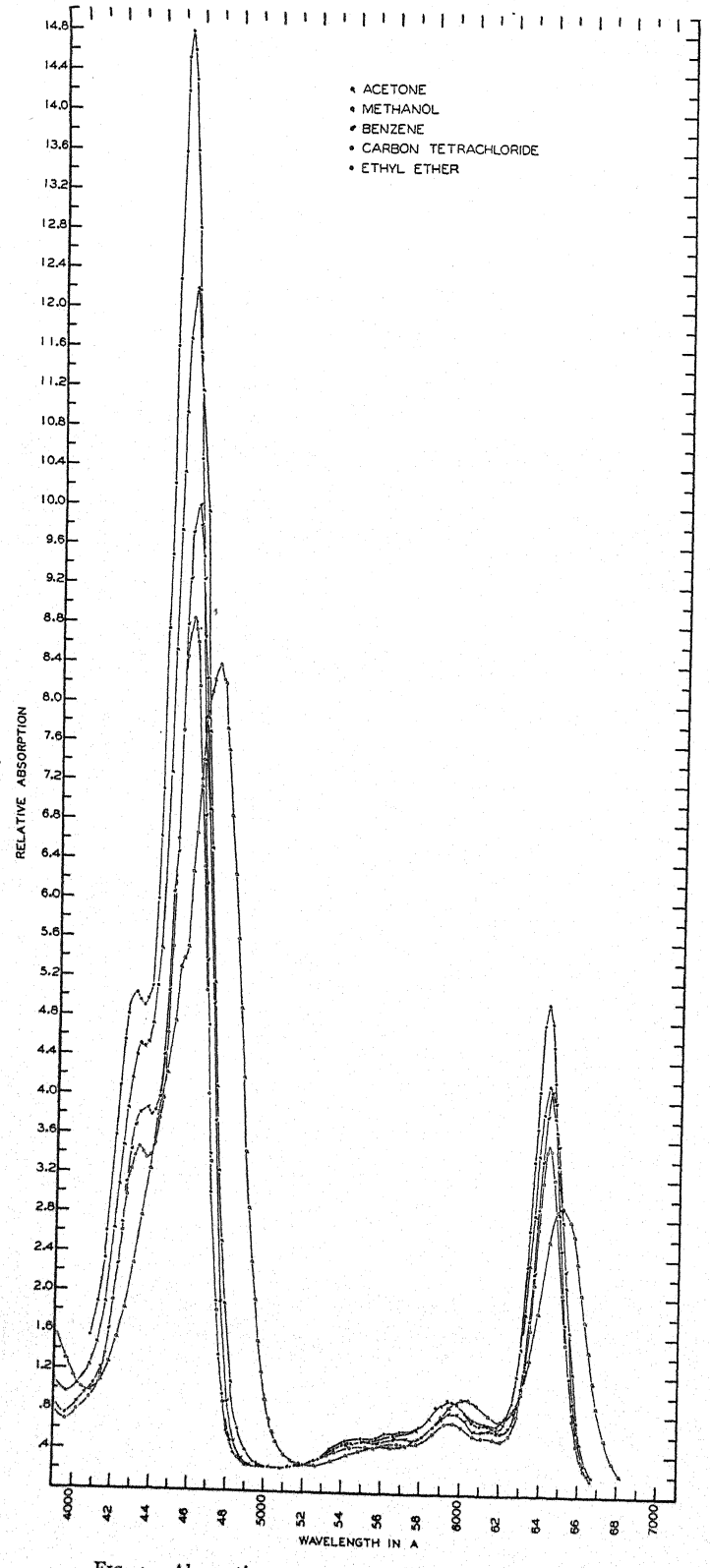


FIG. 4.—Absorption spectra of chlorophyll *b* solutions

In this comparison of the spectra in different solvents, no use is made of specific absorption coefficients, designated in other work from this laboratory by "$\propto$." However, the R values of tables 1 and 2 calculated from the relative absorption will have the same numerical values as though they had been derived from specific absorption coefficients, since the validity of Beer's law is the basis of all these comparisons. For brevity, no distinction is made between the R values calculated here and those defined earlier (29). A similar value, R'_a, for the blue maximum permits a useful comparison. The ratio of R'_a to R_a expresses the ratio of absorption intensity at the blue maximum to that at the red maximum. These calculations are presented in table 1.

TABLE 1

ABSORPTION MAXIMA OF CHLOROPHYLL *a* IN VARIOUS SOLVENTS

Solvent	Index of refraction (20° C.)	Major red maximum (A)	Minor red maximum (A)	Major blue maximum (A)	Minor blue maximum (A)	R_a*	R'_a†	$\frac{R'_a}{R_a}$
Methanol	1.33	6640	6165	4340	4180	45.0	45.1	1.00
Ethyl ether	1.35	6600	6140	4290	4100	52.7	70.2	1.33
Acetone	1.36	6615	6155	4315	4120	43.9	55.2	1.26
Isopropyl ether	1.37	6610	6145	4300	4115	44.6	57.5	1.29
2-methyl propanol-1	1.39	6645	6175	4330	4150	35.2	36.1	1.02
Butanol-1	1.40	6650	6175	4325	4155	39.4	40.7	1.03
1, 4-dioxane	1.42	6610	6215	4330	4110	48.3	70.7	1.46
Cyclohexane	1.43	6600	6160	4295	4100	33.9	46.1	1.36
2-ethyl hexanol-1	1.43	6665	6190	4340	4160	48.0	48.2	1.00
Methyl oleate	1.46	6620	6160	4315	4115	41.3	51.5	1.25
Carbon tetrachloride	1.46	6635	6180	4325	4135	29.3	38.6	1.32
Olive oil	1.48	6645	6200	4330	4140	43.0	55.5	1.29
Benzene	1.50	6640	6175	4325	4135	39.5	52.7	1.33

* $R_a = \frac{\propto \text{ red maximum}}{\propto 5050}$. † $R'_a = \frac{\propto \text{ blue maximum}}{\propto 5050}$.

TABLE 2

ABSORPTION MAXIMA OF CHLOROPHYLL *b* IN VARIOUS SOLVENTS

Solvent	Index of refraction (20° C.)	Major red maximum (A)	Minor red maximum (A)	Major blue maximum (A)	Minor blue maximum (A)	R_b*	R'_b†	$\frac{R'_b}{R_b}$
Methanol	1.33	6510	6025	4710	4540	11.3	32.2	2.85
Ethyl ether	1.35	6425	5940	4530	4285‡	18.9	56.2	2.98
Acetone	1.36	6435	5950	4560	4315	15.9	46.9	2.95
Carbon tetrachloride	1.46	6440	5950	4570	4320	13.4	34.0	2.54
Benzene	1.50	6450	5965	4590	4350	15.7	38.4	2.45

* $R_b = \frac{\propto \text{ red maximum}}{\propto 5200}$. † $R'_b = \frac{\propto \text{ blue maximum}}{\propto 5200}$.

‡ Attention is called to a typographical error of 15 A in the position of this maximum as reported earlier (29).

Similar data for component *b* in five solvents are presented in table 2.

CRITERIA OF PURITY

The spectroscopic criteria of purity presented by ZSCHEILE and COMAR for ether solutions (29) were used in this

study. A small portion of each preparation was eluted from the column with ethyl ether and tested to establish the purity of the chlorophyll preparation as a whole. For chlorophyll *a* (experiment 7), R_a was 53.8 in ether. This preparation was made from corn leaves and was studied in methanol, acetone, isopropyl ether, cyclohexane, carbon tetrachloride, and benzene solutions. Component *a* (experiment 2), from spinach leaves, was studied in 1, 4-dioxane, 2-methyl propanol-1, butanol-1, 2-ethyl hexanol-1, methyl oleate, and olive oil. Its R_a value was 46.8 in ether. This is not as good a preparation as the other, but time did not permit a repetition on a better preparation. Therefore significance should not be attached to very small spectroscopic differences between curves for solutions of these two preparations of component *a* in correspondingly different solvents because of the difference in purity.

For preparation 8, used in the ultraviolet study, R_a was 52.6 and R_b was 18.7 in ether, which is in good agreement with preparations studied previously (29). In the case of preparation 10, higher values were obtained. These ratio values were measured by two methods: (1) with the customary 1-cm. cell and appropriate dilutions of 51- and 11-fold for *a* and *b* respectively; and (2) with the same concentration throughout but using a cell length of 1 cm. for the wavelengths 6600 and 6425 A and 12 cm. for wavelengths 5050 and 5200 A. The solutions employed are thus quite dilute. The log values ranged from 0.81 to 0.18 in the case of *a* and from 0.30 to 0.52 for *b*, the latter being optimum and the former only slightly outside the working range customarily used in this laboratory (29). The R_a values obtained by methods (1) and (2) were 53.4 and 53.0, respectively, and the corresponding R_b values were 19.2 and 19.5. These R_a values agree well with the highest value of 53.3, reported earlier (29) from a corn leaf sample. These R_b values are *ca.* 5 per cent higher than the average of three earlier values (29). ZSCHEILE, COMAR, and MACKINNEY (30) recently reported absorption values obtained on a dried sample of chlorophyll *b* prepared by MACKINNEY, from which R_b values of 18.0–18.2 may be calculated. These two preparations were made from spinach leaves. All absorption spectra of figure 4 are of chlorophyll *b* (experiment 8). In calculating the concentrations from absorption values at the red maxima, specific absorption coefficients of 103.0 (6600 A) and 58.0 (6425 A) for *a* and *b*, respectively, were used for preparation 10 because of the higher ratio values, instead of corresponding average values of 102.1 and 56.8 (29) as used for preparation 8. The difference between the R values found for preparation 10 and earlier values was thus arbitrarily divided between a possible increase in the red and a decrease in the green regions, since a definite decision on the exact cause of the increased R values was not possible.

DRIED CHLOROPHYLL

An effort was made to dry chlorophyll *a* very rapidly in the absence of a glass surface by evaporation of a small stream of an ether solution ($R_a = 51.1$) injected into an evacuated glass chamber through a capillary tube. Some solution struck the sides of the vessel and therefore some evaporation occurred from the glass surface, which previous work indicated should be minimized (29). An R_a value of 42.9 was observed immediately after drying in this manner.

During the early part of a certain preparation of chlorophyll *a* from corn seedlings, an unusual tendency was noted

for the chlorophyll to precipitate.[4] Precipitation started after the first washing of the petroleum ether solution with 80 per cent aqueous methanol and was almost complete after the third washing. After filtration and chromatography on sucrose, the phase test on the chlorophyll of the lower portion of the column indicated the presence of carotenoids. After solution in an ether-petroleum ether mixture, it was washed repeatedly with 80 per cent methanol and finally with 90 per cent methanol. Following two washings with water, the chlorophyll *a* precipitated in leaflike flakes of unusually large size. A portion of the material was removed without drying, dissolved in ether, and tested spectroscopically. The R_a value was 53.0. After drying under reduced pressure for 1 hour, an R_a value of 55.7 was observed; after further drying under high vacuum, the R_a value had decreased to 53.9, indicating a small increase rather than a decrease on drying. This was unusual in our experience, since many previous efforts to dry chlorophyll with retention of a high R_a value had failed (29). The ratio of the maximum at 6600 A to that at 4100 A was identical with the average reported (29) on undried samples. The cause for the increase of R_a on drying this sample is not known. This sample (experiment 6) was small in amount and permitted only limited study of its properties during cold storage. After 9 weeks, the R_a value of the dried portion had decreased to 48.9, whereas a portion kept at the same temperature in ether solution still had a very high R_a value, 54.0.

It is thus possible to dry chlorophyll *a* with retention of the spectroscopic properties it had before drying (at least so far as indicated by the R_a value). The optimum conditions have not been clarified, however, and rigid spectroscopic control should always be relied upon as a criterion of purity. In our experience chlorophyll is most stable when kept in ether solution at a low temperature. Values of $\frac{R_a'}{R_a}$ for ether solutions of a dried sample prepared by MACKINNEY and studied in this laboratory (30) approach very closely the value reported here.

The addition of a small amount of hydrogen peroxide to an ether solution of chlorophyll *a* ($R_a = 51.1$) reduced the R_a value to 46.0 after 2 hours. The ratio of the absorption at 4290 to 6600 A checked average values reported earlier (29). The absorption at 6600 A was unchanged but that at 5050 A was increased 11.7 per cent, causing the observed decrease in R_a. This change was not due simply to the effect of the acidic condition, since absorption at 6600 A would then have decreased.

ULTRAVIOLET SPECTRA

The ultraviolet absorption data are plotted in figure 2, as the specific absorption coefficient (29) versus wavelength. The concentrations were determined spectroscopically at the red maxima on solutions eluted directly from the adsorption columns. Maxima or shelves in the curves were found at the following wavelengths: 2820, 2960, 3120, 3260, and 3800 for chlorophyll *a;* 2900, 3060, 3250, 3340, 3570, and 3750 for chlorophyll *b*. Minima have wavelengths as follows: 2730, 3380, and 3880 A for component *a;* 2740, 3180, and 3930 A for component *b*.

Discussion

The group of solvents in which chlorophyll *a* was studied includes three ethers, four alcohols, one ester, a mixture of

[4] A similar tendency for early precipitation was observed previously by C. L. COMAR in the case of a preparation from spinach made in this laboratory.

esters, aromatic solvents (both saturated and unsaturated), a ketone, and one with a very symmetrical molecule, carbon tetrachloride. These solvents cover a considerable range of polarity (as indicated by the dielectric constants) and refractive index, as well as other physical properties such as boiling point, specific gravity, and internal pressure. Significant correlations with spectroscopic properties could be found only with the first two of these physical properties, discussed in the following paragraphs.

KUNDT'S RULE

Kundt's rule, that the absorption band of a solute dissolved in a colorless solvent of high refractive index is shifted toward longer wavelengths compared with the corresponding absorption band of the same solute in another colorless solvent of lower refractive index, has often been studied in relation to the red maximum of chlorophyll (3, 6, 10, 12, 22). Certain controversial and historical aspects of this question have been discussed by MACKINNEY (12, 13).

CHLOROPHYLL *a*.—From table 1 the following conclusions may be drawn, considering all the bands listed:

1. When all solvents are considered together, no correlation exists between position of maxima and refractive index of the solvent.

2. Among the four alcohols studied, the red maxima follow Kundt's rule; the blue maxima do not.

3. Among the ethers, differences are small but not contradictory to Kundt's rule, within the limits of error.

4. The rule is followed between ethyl ether and acetone, but if isopropyl ether is included in the comparison the rule is not obeyed.

5. Among the two hydrocarbons, cyclohexane and benzene, all four maxima follow the rule.

6. Among the ester solvents, methyl oleate and olive oil, all four maxima follow the rule.

It may be noted that when two solvents with the same refractive indices are compared, as cyclohexane with 2-ethyl-hexanol-1, and methyl oleate with carbon tetrachloride, great differences occur between the wavelengths of corresponding maxima. In some cases this difference is equal to the entire range found for the thirteen solvents studied. With such pairs of solvents, great differences exist in their chemical nature. With such a pair as 2-methyl propanol-1 and butanol-1, with nearly the same refractive index but similar chemical properties, wavelengths of maxima are essentially identical.

CHLOROPHYLL *b*.—The more limited number of solvents in which component *b* was studied prevents comparisons as extensive as those made for component *a*. With the exception of methanol, Kundt's rule is obeyed fairly well, although differences between acetone and carbon tetrachloride are very slight and not so great as was the case for component *a*. Comparisons between ethyl ether and acetone are similar for both components. Differences between carbon tetrachloride and benzene are greater than was the case for chlorophyll *a*. These two solvents have about equal dielectric constants. No better correlations exist between dielectric constant and wavelengths of maxima than was the case for component *a*.

Discrepancies in wavelengths prevent a good comparison with BIERMACHER'S work (3) on chlorophyll *a*, but in the case of the *b* component his data agree with those presented here so far as relative positions of maxima are concerned.

The conclusion of HUBERT (10) that the band shifts to shorter wavelengths in polar media is not substantiated for the case of methanol in comparison with ether, acetone, and benzene. In general the data for the four alcohols agree with the observations of EGLE (6), that in comparison with other solvents studied

the bands occur at longer wavelengths than would be expected from Kundt's rule. The relationship between ethyl ether and benzene solutions also agrees with his report. Certain causes for disagreement among various workers on this subject have been discussed previously (27).

Thus, the statement of WAKKIE (22) that Kundt's rule is obeyed when restricted to substances which have certain hydrophilic groups in common is upheld to a limited extent; it is also evident that Kundt's rule cannot be applied to chlorophyll solutions in general. The limited data presented here are favorable to Kundt's rule only when applied to solvents of similar chemical nature.

ANALYTICAL APPLICATIONS

The ratio values of tables 1 and 2 and the absorption curves of figures 1, 3, 4 permit numerous comparisons having analytical applications. In comparing the ratios of the absorption values at the blue maxima with those at the red maxima, a clear distinction exists for chlorophyll *a* between the four alcohols as a group and the remaining solvents. In the alcoholic solutions the blue and red maxima are of equal heights, whereas in all the other solvents the blue maximum is considerably higher than the red. The data for methanol were obtained on a preparation different from that used for the other three alcohols. This similarity between alcoholic solutions would thus appear not to be a property peculiar to a single preparation.

For methanol solutions MACKINNEY (15) and STRAIN and MANNING (21) reported a slightly higher (*ca.* 4 per cent) absorption at the red maximum of component *a* than at the blue maximum. The relative height of the lowest minimum for methanol agrees better with STRAIN and MANNING'S (21) curve for component *a* but better with MACKINNEY'S data (15) for component *b*. There are great discrepancies between results reported here and those of RABINOWITCH and WEISS (18), not only in relative heights of bands but also in wavelengths of maxima and presence or absence of bands. The $\frac{R'_a}{R_a}$ and $\frac{R'_b}{R_b}$ values calculated from MACKINNEY'S data (15) are 0.96 and 2.82, respectively, compared with corresponding values of 1.00 and 2.85 of tables 1 and 2. These data indicate good agreement, but differences in wavelength in the blue region throw some doubt on the validity of this comparison. Uncertainty arises because MACKINNEY has reported (15) wavelengths as "near maximum."

Corresponding values for anhydrous ether solutions calculated from previous data (29) are 10 per cent higher than those from MACKINNEY'S values (15). Here again, wavelengths may not be directly comparable for the blue region, although agreement is excellent in the red.

For acetone solutions the values of $\frac{R'_a}{R_a}$ and $\frac{R'_b}{R_b}$ agree well with those calculated from MACKINNEY'S data (14). Values from his data vary with source as follows: $\frac{R'_a}{R_a}$ from 1.22 to 1.30, average 1.26; $\frac{R'_b}{R_b}$ from 2.61 to 2.98, average 2.75. Corresponding values reported here are 1.26 and 2.95, respectively.

The curves for benzene solutions do not agree with those determined by HAUSSER (7) and WINTERSTEIN and STEIN (24) when the relative heights of maxima are considered. HAUSSER'S curve for component *a* has an extra band near 5500 A. The ratios of blue to red maxima reported here are considerably lower than those of WINTERSTEIN and STEIN (24) and closer to those calculated from data

of MACKINNEY (14). The wavelengths of maxima are in good agreement with those reported by MACKINNEY (14).

In the case of component *b*, the solutions with solvents containing oxygen have higher values of $\frac{R_b'}{R_b}$ than those of the other two solvents, which is not true with the values of $\frac{R_a'}{R_a}$. The blue region is of importance to characterize the components but has less analytical importance than the red region because of the interference of carotenoids in most practical applications.

In spectrophotometric analysis it is generally desirable to choose a solvent in which the substance has the most sharp, intense, and well-defined absorption bands, although other considerations are also necessary for practical reasons. Sharp intense bands increase the sensitivity of analysis and facilitate more accurate tests for identification and purity of samples. Ethyl ether has been used in analytical applications (5, 4), and it is clear that the R_a and R_b values for this solvent are higher than for other solvents examined, although several others have values of comparable magnitude. The R_a' and R_b' values are also very high. Corresponding values of R_a and R_a' for 1, 4-dioxane are nearly the same.

The curve for component *a* in 1, 4-dioxane, although having very sharp major maxima in the blue and red regions, does not resolve the band near 5850 A clearly. The band at 4110 A is most clearly resolved in this solvent. The spectra of acetone solutions do not have as sharp bands as those of ethyl ether solutions, although acetone is not an unfavorable solvent from this viewpoint. Aqueous acetone has been used as an analytical solvent (15). Among the alcohols studied, 2-ethyl hexanol-1 has the highest values of R_a and R_a'. In general, spectra of the alcoholic solutions do not have the minor bands well resolved. Spectra of methanolic solutions of both components are particularly deficient in structural detail.

ULTRAVIOLET SPECTRA

The ultraviolet region was not studied as intensively as the visible region because its analytical importance is minor, effects of contaminants are greater, and it has little application to photosynthesis compared with the visible region. The general characteristics of the ultraviolet curves agree well in wavelength with those found earlier for chlorophylls *a* (26, 28) and *b* (26). The ultraviolet spectra determined by HAUSSER (7) and by WINTERSTEIN and STEIN (24) for benzene solutions are lacking in structural detail but have somewhat the same general shape as the curves reported here for ether solutions.

Summary

1. Solutions of chlorophyll *a* were prepared in thirteen solvents and of chlorophyll *b* in five solvents by direct elution from sucrose adsorption columns.

2. Absorption spectra for each solvent are reported as measured spectrophotoelectrically in the visible region.

3. Spectra for the ultraviolet region to 2650 A were determined for solutions in ethyl ether.

4. A preparation of dried chlorophyll *a* was made which retained excellent spectroscopic properties.

5. The application of Kundt's rule to chlorophyll solutions is discussed, as well as certain relations of spectroscopic properties and solvents to analytical applications.

It is a pleasure to acknowledge the aid of C. L. COMAR in the first preparation employed in this work, that of ARTHUR M. BRUNSON and RALPH LINCOLN in supplying the corn seedlings as a source of part of the chlorophyll, and the assistance of L. MCALISTER in many of the calculations.

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LITERATURE CITED

1. ARMSTRONG, E. F., ALLEN, J., and MOORE, C. W., The fatty acid constituents of some natural fats. I. The oils from the coconut. Jour. Soc. Chem. Ind. 44:63t–68t. 1925.
2. BASKERVILLE, C., and HAMOR, W. A., The chemistry of anaesthetics. I. Ethyl ether. Jour. Ind. Eng. Chem. 3:378–398. 1911.
3. BIERMACHER, O., The visible and infra-red fluorescence spectra of chlorophyll *a*, chlorophyll *b*, and several porphyrins. Thesis, Univ. Fribourg, Switzerland. 1936.
4. COMAR, C. L., Analysis of plant extracts for chlorophyll *a* and *b* using a commercial spectrophotometer. Ind. Eng. Chem. Anal. Ed. 14: 877–879. 1942.
5. COMAR, C. L., and ZSCHEILE, F. P., Analysis of plant extracts for chlorophylls *a* and *b* by a photoelectric spectrophotometric method. Plant Physiol. 17:198–209. 1942.
6. EGLE, K., Über die Gültigkeit der Kundt'schen Regel bei Chlorophyllspektren. Sitzungsb. Heidelb. Akad. Wiss. 1:19–29. 1939.
7. FISCHER, H., and STERN, A., Die Chemie des Pyrrols. II. Band. Pyrrolfarbstoffe 2. Hälfte. Akad. Verlag. M.B.H. 1940.
8. HOGNESS, T. R., and POTTER, V. R., Spectrometric studies in relation to biology. Annual Rev. Biochem. 10:509–530. 1941.
9. HOGNESS, T. R., ZSCHEILE, F. P., JR., and SIDWELL, A. E., JR., Photoelectric spectrophotometry. An apparatus for the ultraviolet and visible spectral regions: its construction, calibration, and application to chemical problems. Jour. Phys. Chem. 41:379–415. 1937.
10. HUBERT, B., The physical state of the chlorophyll in the living plastid. Rec. Trav. Bot. Néerl. 32:323–390. 1935.
11. KRAYBILL, H. R., BREWER, P. H., and THORNTON, M. H., Processes of producing an adsorbent agent. U.S. Patent no. 2.174,177. 1939.
12. MACKINNEY, G., Applicability of Kundt's rule to chlorophyll. Plant Physiol. 13:427–430. 1938.
13. ———, Kundt's rule. Plant Physiol. 15:359. 1940.
14. ———, Criteria for purity of chlorophyll preparations. Jour. Biol. Chem. 132:91–109. 1940.
15. ———, Absorption of light by chlorophyll solutions. Jour. Biol. Chem. 140:315–322. 1941.
16. MEYER, K. P., Spektrometrische Untersuchungun über den Zustand des Chlorophylls in der Pflanze, in Extrakten und Reinpräparaten. Helvetica Physica Acta 12:349–393. 1939.
17. MUNCH, R. H., A gaseous discharge tube especially designed as an intense source of continuous ultraviolet radiation. Jour. Amer. Chem. Soc. 57:1863–1865. 1935.
18. RABINOWITCH, E., and WEISS, J., Reversible oxidation of chlorophyll. Proc. Roy. Soc. London (A) 162:251–267. 1937.
19. ROTHEMUND, P., Private communication. 1941.
20. SMITH, E. L., Solutions of chlorophyll-protein compounds (phyllochlorins) extracted from spinach. Science 88:170–171. 1938.
21. STRAIN, H. H., and MANNING, W. M., Chlorofucine (chlorophyll γ), a green pigment of diatoms and brown algae. Jour. Biol. Chem. 144:625–636. 1942.
22. WAKKIE, J. G., Notes on the possible structure of the chlorophyll-granules in the plastid. Proc. Kon. Akad. Const. 38:1082–1086. 1935.
23. WEISSBERGER, A., and PROSKAUER, E., Organic solvents. Physical constants and methods of purification. English transl. by RANDAL, G. A., from the German. Clarendon Press, Oxford. 1935.
24. WINTERSTEIN, A., and STEIN, G., Fraktioniering und Reindarstellung organischer Substanzen nach dem Prinzip der chromatographischen Adsorptionsanalyse. Zeit. Physiol. Chem. 220:247–277. 1933.
25. ZSCHEILE, F. P., An improved method for the purification of chlorophylls *A* and *B;* quantitative measurement of their absorption spectra; evidence for the existence of a third component of chlorophyll. BOT. GAZ. 95:529–562. 1934.
26. ———, Unpublished work. 1936.
27. ———, Plastid pigments, with special reference to their physical and photochemical properties and to analytical methods. Bot. Rev. 7:587–648. 1941.
28. ZSCHEILE, F. P., and COMAR, C. L., Unpublished work. 1939.
29. ZSCHEILE, F. P., and COMAR, C. L., Influence of preparative procedure on the purity of chlorophyll components as shown by absorption spectra. BOT. GAZ. 102:463–481. 1941.
30. ZSCHEILE, F. P., COMAR, C. L., and MACKINNEY, G., Interlaboratory comparison of absorption spectra by the photoelectric spectrophotometric method. Determinations on chlorophyll and Weigert's solutions. Plant Physiol. 17:666–670. 1942.

CYTO-GENETIC CONSEQUENCES OF X-RAY TREATMENT OF POLLEN IN PETUNIA

CHARLES M. RICK

Introduction

Artificial stimulation of the haploid egg is attracting ever-increasing interest. The usefulness, both economic and theoretical, of a dependable method for producing haploid organisms, has been pointed out by EAST (6), BLAKESLEE (2), and others. The possibilities of attaining this goal by the use of chemicals are already being explored (23).

Some investigators have succeeded in raising the frequency of haploids by various techniques. Certain of these methods have also yielded maternal diploids which are suspected of having originated by doubling of the chromosome number in a haploid egg. In this way pure lines would be produced with greater ease than by way of haploids. No attempt is made here to review all the pertinent literature, since IVANOV (10) has adequately surveyed the subject up to 1938. Polyembryony has been the source of a great number of plant haploids (24), but their production by this technique is naturally limited to those species in which there is a fair frequency of multiple seedlings, including haploids. Interspecific hybridization also accounts for many haploids. JØRGENSEN (12) reported the high yield of 20 per cent haploids in thirty-five plants produced by the cross *Solanum nigrum* ($2n = 72$) × *S. luteum* ($2n = 48$). KIHARA (14) found 37.5 per cent haploids in *Triticum monococcum* from flowers emasculated 9 days before the application of normal pollen, but this figure was based on a population of only eight plants.

The use of X-rayed pollen was found by KATAYAMA (13) to yield 17.6 per cent haploids (in a total of ninety-one plants) in a line of *T. monococcum* which without treatment produced only 0.48 per cent haploids. In the varieties *humilis* and *texana* of *Nicotiana rustica*, IVANOV (10) secured four haploids and seven maternal diploids by means of X-rayed pollen. The failure of X-rayed pollen to stimulate the production of haploids in *Datura* is reported by BLAKESLEE (2). The results of similar treatment in the present experiment were negative. Certain aspects of this study of the cytologically aberrant plants resulting from the X-ray treatment have already been published in abstract form (20).

STEBBINS (21) has pointed out the frequent misuse of the term parthenogenesis in this type of experiment. In the functional sense parthenogenesis implies natural development of the egg without external stimulation. It is more accurate to designate as haploid, or reduced pseudogamy, cases where development of the haploid egg is a response to the activity of pollen which is incapable of fertilization. It is possible, as already mentioned, that some external stimulus might cause the doubling of the egg chromosomes and the subsequent development of a diploid embryo. In this paper the term haploid-diploid pseudogamy is arbitrarily chosen to indicate this phenomenon.

Investigation

HAPLOID PSEUDOGAMY

Anthers of *Petunia* were collected immediately before anthesis and stored in gelatine capsules. The source of X-rays was a Wappler clinical unit operated at 200 kv. and 10 ma. Filters of $\frac{1}{4}$ mm. copper and $\frac{1}{4}$ mm. aluminum were employed. All anthers were irradiated in capsules at a distance of 10 cm. from the

tungsten anticathode, at which distance the intensity of irradiation was approximately 800 r per minute. By varying the lengths of exposure, doses from 10,000 to 100,000 r were achieved. Shortly after this treatment, the pollen was applied to stigmas of previously emasculated flowers. Simultaneously some stigmas were dusted with untreated pollen and others were left unpollinated. Approximately forty flowers were pollinated at each dose; a total of about 700 pollinations was made. Seeds from all of these treatments were harvested, and, starting with lots from pollen which had received 100,000 r and proceeding toward less severe treatments, seeds were sown and plants grown to maturity for further study. All sowings were made on nutrient agar under aseptic conditions in order to carry through the tender seedling stage as many as possible of the weaker, more aberrant types.

If by means of this treatment the haploid egg cell were to be stimulated to develop pseudogamously, it would give rise to a haploid plant. Accordingly all plants produced by pollen surviving the highest doses were examined cytologically. This search for haploids was accomplished by examination of metaphase chromosome figures in root tips, which of course would also reveal any gross chromosomal aberrations. The root tips were fixed, imbedded in paraffin, sectioned, and stained with iodine-crystal violet in the usual fashion. RANDOLPH'S method (18) of mounting root tips was found excellent for this purpose.

Prior to germination, no satisfactory criterion of the most effective X-ray dose could be found. Even pollen which had received 100,000 r stimulated considerable development of the ovary and production of some seeds, which, although inviable, were very difficult to distinguish from viable seeds. Furthermore, there was great fluctuation in the number of seeds produced and in the size of the mature fruit.

As noted in table 1, pollen could effect fertilization after doses as high as 50,000 r. There can be little doubt that treated pollen functioned here, since one of the plants produced showed a typical X-ray-induced chromosomal aberration. Although the results were too diverse to permit the establishment of a relation between dose and germination, it is possible to estimate the half-kill doses. Germination was reduced to half the level of untreated material at about 16,000 r. The ratio of the numbers of plants produced per flowers pollinated was reduced to half at approximately 9,000 r. The highest frequency of cytologically aberrant plants, 18.5 per cent, was produced by pollen which had been exposed to 30,000 r.

No haploids were found in the sample of 378 plants whose root tips were examined. This sample included all plants from the 25,000 to 100,000 r lots, and some of the plants from lower doses, which survived until time of chromosome examination.

TABLE 1

SUMMARY OF X-RAY EFFECTS

Source of pollen	No. of flowers pollinated	Germination (%)	Mean no. plants per pollination	Diploid	Aberrant*	Aberrant (%)
(Not pollinated)	25	.....	.....	.....	.....	.....
P117 untreated	61	53.3	64	All	.....	.....
P117 10,000 r	30	40.2	28.7	32	1	3.1
P117 20,000 r	35	17.7	13.2	67	9	11.8
P117 25,000 r	36	14.1	6.4	121	15	11.6
P117 30,000 r	61	7.0	0.82	22	4	18.5
P117 40,000 r	66	8.7	0.5	13	1	7.1
P117 50,000 r	69	4.9	0.32	12	1	7.7
P117 60,000 r	31	2.9	0.1	2	.....	0.0
P117 75,000 r	32	2.4	0.1	12	.....	0.0
P117 100,000 r	29	0.0	0.0	.....	.....	.....

* Under aberrant are listed the following types: $2n = 13$, $13 + 1f$, $14 + f$, $13 + 2f$, $12 + 2f$.

The failure of haploid plants to appear in this sample might be attributed to failure of the treatment to induce haploid pseudogamy. No haploids in *Petunia* have been reported in the literature, in spite of the frequent use of this genus in cyto-genetic research. If the frequency of natural occurrence of haploids can be taken to mean anything in regard to their artificial production (and this is borne out in the case of *T. monococcum*), then it is not unusual that no haploids were obtained in this experiment. Haploid-diploid pseudogamy may have occurred, thereby throwing all potential haploids into the diploid group through doubling of the chromosome number, although further results—discussed later—shed considerable doubt on this hypothesis. The other possibilities—that haploids were produced but died in early seedling stages or that the sample was too small to reveal any induced haploidy—obviously cannot be ruled out.

HAPLOID-DIPLOID PSEUDOGAMY

Diploids arising by haploid-diploid pseudogamy would be completely homozygous, and their progeny (by selfing) could be readily distinguished from the more variable progeny of diploids of any other origin when the X-rayed pollen has been applied to stigmas of plants heterozygous for a considerable number of characters. To reveal this mode of origin, the following plan was adopted as shown on opposite page.

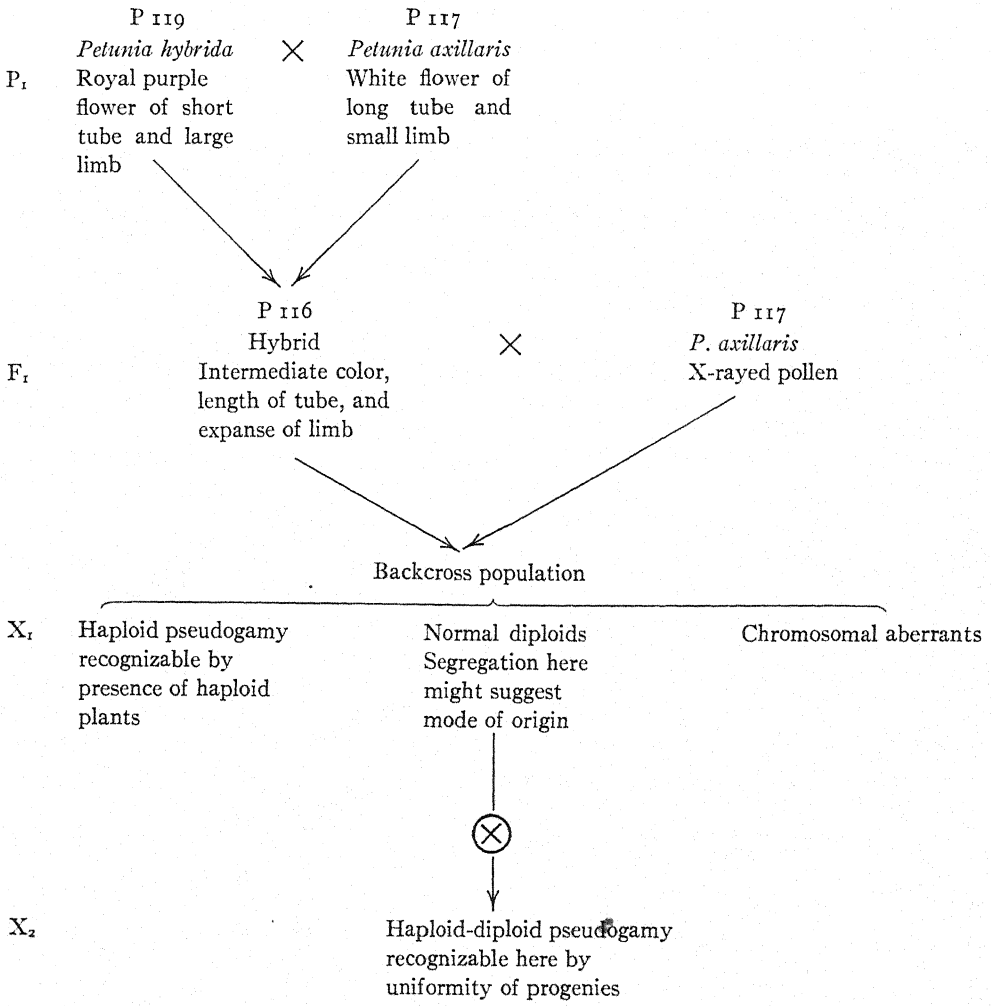
A somewhat similar plan was followed by EAST (7). Plants of *Fragaria vesca* heterozygous for two Mendelian factors were pollinated by *F. virginiana* and *F. chiloensis*. All plants obtained from this cross were typical *F. vesca*. Ratios obtained for the two factors were 19:5 and 16:12, suggesting that some individuals might have been produced by haploid-diploid pseudogamy. A more rigorous proof might have been obtained by selfing these plants and testing the next generation for uniformity, as proposed in the present experiment; but this EAST did not attempt owing to "certain technical difficulties."

In the experiments of EAST (7) and IVANOV (10), the pollen used to induce pseudogamy carried factors which would be manifest in the phenotypes of hybrid offspring. Thus it was possible to distinguish between true hybrids and maternal diploids. However, in neither case could a separation be made in the matroclinous group between those resulting from self-pollination and those resulting from apomixis—presumably haploid-diploid pseudogamy. Although the present experiment would have been improved by the use of X-rayed pollen carrying factors which would identify hybrid individuals in the offspring, an adequate pollen source of this type was not available.

Many genetic differences exist between P 119 and P 117, the parents of the hybrid used as receptor of the X-rayed pollen. The presence or absence of anthocyanin pigment in the limb of the corolla of these lines is determined by a single gene pair, according to results obtained in this study and to FERGUSON (8). In addition to this basic difference, an undetermined number of supplementary genes exist which modify the color variously in a range from pale lilac to deep royal purple. Variation was also noted in the coloration of the throat of the corolla. Male-sterility, a simple recessive character, also appeared in some of the F_2 populations. The differences in dimension of the flower corolla were inherited in the expected quantitative fashion. Of these dimensions, length of

tube and diameter of limb were most conveniently measured. Many other differences of a less tangible nature exist between P 119 and P 117.

All applications of the X-rayed pollen were made in the field, where every possible precaution against self-pollination and contamination was observed. The failure of the twenty-five unpollinated flowers to set any seed attests to some degree the conditions of the experiment. Yet, in view of the great number of pollinations attempted, occasional contamination could hardly be avoided. This was anticipated in the design of the experiment, which attempted to distinguish only completely homozygous individuals from those of hybrid origin, whether by X-rayed pollen or contamination. Nevertheless, the type of segregation obtained frequently indicated the type of hybrid origin. Contamination was encountered, and—especially when it occurred in the higher doses—it obscures the relation between X-ray dose and frequency of induced aberration.



For example, among the thirty-two ovaries developed after application of pollen treated with 75,000 r, none produced viable seed except one which gave rise to 14 plants—a fact which in itself suggests contamination. Of these fourteen, eleven survived to flowering; all were diploid; ten had colored flowers, one had white flowers. Obviously this could not represent a backcross family or a family arising out of haploid-diploid pseudogamy, for in either case a 1:1 ratio is expected. By noting segregations in other suspiciously large families, certain other diploids were dismissed from consideration as possible homozygotes.

A sample of forty-five diploids was selected for the tests of homozygosity. These included all diploids produced by pollen receiving doses of 40,000 to 75,000 r; also four families in the 25,000 and 30,000 r lots, each of which included some aberrant individuals and showed the expected 1:1 segregation for flower colors. Nineteen of the forty-five diploids were ruled out as contaminants because of aberrant segregation, as in the case of the 75,000 r lot already described.

The remaining twenty-six plants were self-pollinated and sample progenies grown for comparison with pure lines, etc., as described in the preceding section. Of these, twenty-two were found to segregate for some readily detectable qualitative character (for example, color of corolla or male-sterility). Dubious cases were reserved for flower measurement. Judging from the type of segregation (for example, those in which white offspring always appeared), the majority of the plants being tested arose from the intended backcross and few from contaminating pollen. The appearance of characters not present in either of the original parents could not be safely relied upon as a criterion of contamination, because some of these might be the expression of mutation induced by the X-radiation. The four remaining populations showed no such tangible variation. But the fact that each of these groups had only white corollas (that is, were homozygous recessive for the color-determining gene) would suggest that they were only the proportion of amphimictic families expected to be homozygous for the qualitative characters observed. This surmise was verified by measurements summarized in table 2.

These four families, together with five families of P 117, the pure line of *P. axillaris*, were grown under comparable conditions in the same field. It will be noted that the average standard deviation for the two dimensions of the lines being tested greatly exceeds that of the pure lines. Where in a few cases the standard deviation of one dimension in a test family does not significantly exceed the highest standard deviation for that character in the pure-line families, the standard deviation of the other dimension in that test family does so. Thus it must be concluded that no evidence of haploid-diploid pseudogamy was encountered in this experiment.

Again, as in the search for haploids, it must be admitted that the failure to discover evidence of haploid-diploid pseudogamy does not prove that it cannot be stimulated by the use of X-rayed pollen in the same material. This phenomenon might have been revealed had more diploids appearing in the lower doses been tested; yet the same relative dosage levels were sampled as those in which IVANOV (10) found maternal diploids. Also, the occurrence of this type of apomixis might be so rare that the same experiment conducted on a much greater scale might be needed to reveal it. Nevertheless, if a technique is to be of any value

in the production of pure lines or haploids for practical or theoretical use, it must certainly yield more of the desired product for the amount of effort involved than IVANOV has obtained.

In the experiments of EAST (7), IVANOV (10), and REDINGER (19), self- and cross-contamination is admittedly a possible explanation of the origin of maternal diploid plants. This was considered by each of these workers but ruled out as unlikely. However, in none of these experiments was incontestable proof offered of the haploid-diploid method of origin. The chromosome condition of the endosperm in such cases might shed some light on the nature of the origin of the diploid embryos. Only in the work of REDINGER (19) was this approach attempted. Here diploid and tetraploid embryos in *Petunia* arose seemingly by haploid-diploid pseudogamy as a result of using pollen of *Salpiglossis sinuata*. Wherever chromosome counts were made in the endosperm of young developing seed, however, they were found to be triploid. If this triploid condition resulted in any other way except by double fertilization in amphimixis, it would certainly be an unusual case.

TABLE 2

SUMMARY OF MEASUREMENTS

Progeny no.	Pedigree	N	Length of tube		Diameter of limb	
			Mean (mm.)	Standard deviation (mm.)	Mean (mm.)	Standard deviation (mm.)
$P_{VI}119$	Inbred	3	40.69	.....	66.26	.....
$P_{IX}117a\text{-}1$	Inbred	15	65.19	0.80 ±0.15	43.79	0.96 ±0.18
$P_{IX}117a\text{-}2$	Inbred	18	64.68	1.47 ±0.25	43.05	1.80 ±0.30
$P_{IX}117a\text{-}3$	Inbred	9	66.05	1.28 ±0.30	44.18	1.54 ±0.36
$P_{IX}117b\text{-}1$	Inbred	17	70.29	0.94 ±0.16	44.66	0.90 ±0.15
$P_{IX}117b\text{-}2$	Inbred	20	68.19	0.69 ±0.11	44.76	1.32 ±0.21
$P_{IX}117$ (mean)	Inbred	79	66.88	1.036	44.09	1.304
$P_{I}116\text{-}5$	F_1 (single clone)	41	54.31	0.57 ±0.06	62.42	1.67 ±0.18
$P_{II}A4$	F_2	84	54.45	3.87 ±0.30	59.51	4.28 ±0.33
$P_{III}N193$	X_2	11	54.78	2.72 ±0.58	50.09	2.07 ±0.44
$P_{III}P103$	X_2	23	60.21	5.22 ±0.77	47.09	3.35 ±0.49
$P_{III}R122$*	X_2	11	61.91	3.39 ±0.72	55.21	2.43 ±0.52
$P_{III}R124$*	X_2	18	61.98	2.40 ±0.40	57.44	2.99 ±0.50
$P_{III}R153$	X_2	15	58.01	3.89 ±0.71	49.36	2.21 ±0.40
Mean	X_2	78	.....	3.52	.....	2.61

* $P_{III}R122$ and $P_{III}R124$ both from selfings of single X_1 plant.

X-RAY–INDUCED CHROMOSOMAL ABERRATIONS

As a byproduct of the present experiment, the cytologically aberrant plants were investigated. From table 1 it can be seen that a total of thirty-one aberrants was detected from examination of root tips. By means of this method only certain types of aberration—for instance, reduction or increase in the number of chromosomes—can be identified with certainty. Translocation might be responsible for some of the changes in length, but in general root-tip figures

would not indicate the amount of translocation or inversion induced by the X-ray treatment.

Most of the plants of the X_1 generation were transferred to the field for observations on their flower characters and for further cytological investigation. For study of the pollen mother cells, buds were fixed in Carnoy's fluid and anthers smeared in iron aceto-carmine. Diakinesis of the first meiotic division proved to be an excellent stage for determining pairing relations and relative sizes of chromosomes (figs. 1, 3, 6).

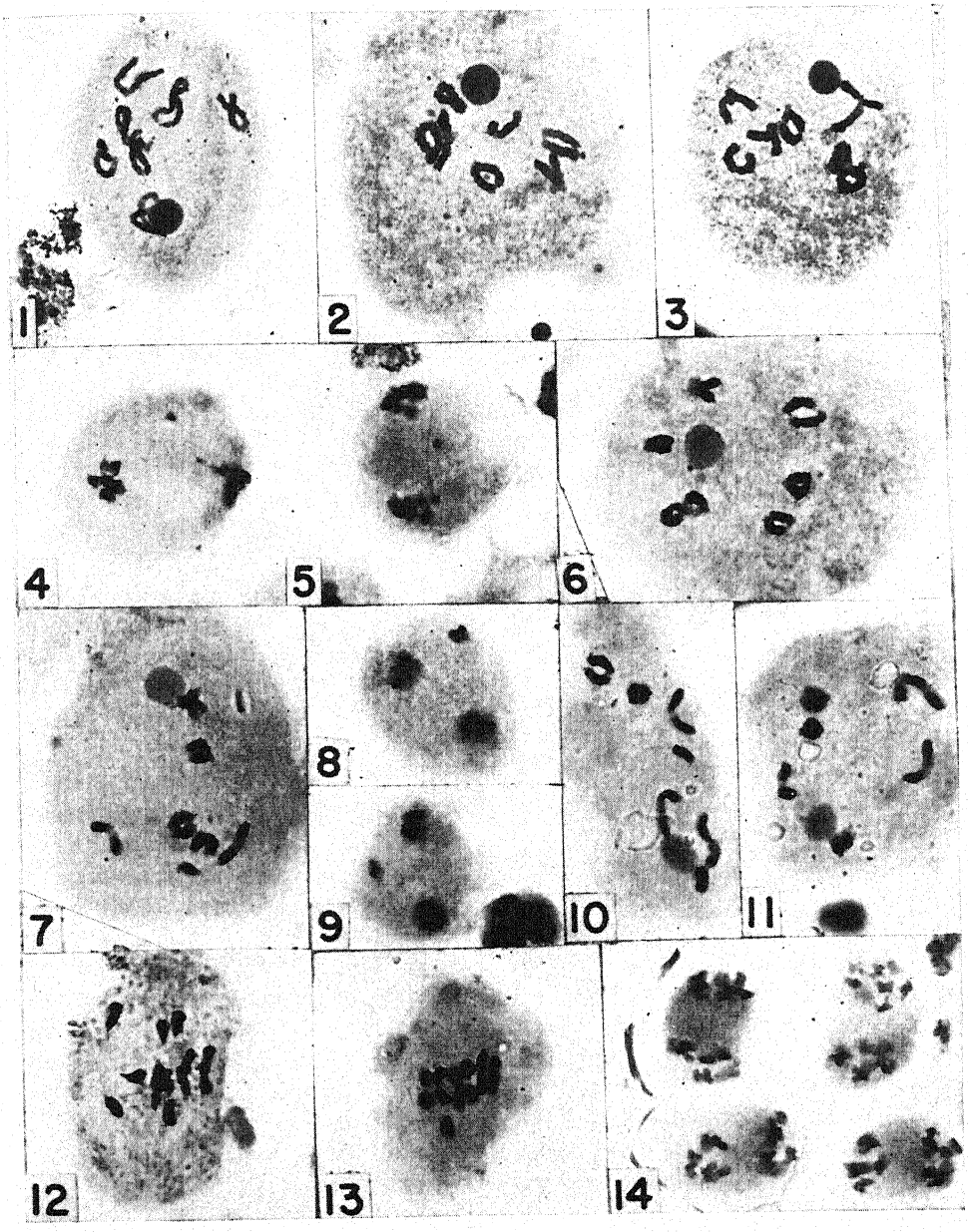
These studies indicated that in many cases the count or morphology of chromosomes in pollen mother cells differed from that in mitotic figures in root tips of the same plant. This situation was definitely established in six plants out of a sample of eighteen which were considered thoroughly enough investigated to reveal a major difference if any existed. This sample of eighteen was not by any means random; in fact, it included only plants which had previously been found aberrant from studies of root tips. It was not possible to make a complete survey of pollen mother cell divisions in all the X_1 generation, so that a random sample could not be attained. It is possible that there were other chimeras, especially of the type in which the root-tip complement was normal and that of the pollen mother cells abnormal.

The chimeras encountered in this experiment seemed to be largely of the type in which the roots as a unit deviated from the tops as a unit, similar to the case of a $2n:2n-1$ chimera in *Zea* reported by McCLINTOCK (17). Evidence of differences within the roots or within the shoots of a single plant is not nearly so abundant or so convincing. Five possible cases in a sample of thirty in root tips and one in sixteen of the pollen mother cells were found. The reservation must be added that in examining the meiotic division of pollen mother cells only the subepidermal layer of the shoots was being sampled. If a periclinal condition was involved in any of the shoots, it would obviously be overlooked. Nevertheless, there was no indication of a periclinal chimeral condition in the root tips. A few diploid-tetraploid sectorial root tips were encountered, but these were probably the result of spontaneous doubling rather than an effect of the X-ray treatment. Such diploid-tetraploid chimeras have been observed in untreated *Petunia* (4, 15).

These intra-plant differences nearly always involved the presence or absence of fragment chromosomes. In two cases the fragment chromosome was lacking in pollen mother cells; in the other four it was lacking in root tips. Thus losses were not limited to root tips, as has been recently observed in *Sorhgum* (11) and in *Paris* (5).

Presumably a fragment chromatid was lost to the cytoplasm in one of the early divisions of the embyro. According to the studies of BHADURI (1) on the embryogeny of *Petunia axillaris*, a chimera of this type could be established by a loss at the second division of the embryo. The chalazal cell resulting from the initial division of the zygote divides to produce two cells, one of which gives rise to the cotyledons and apical meristem, the other to the hypocotyl and the meristem of the radicle. Thus the postulated disturbance would need to be removed by only two mitoses from the time of exposure to X-rays in order to be effective in producing a root-shoot chimera.

The readily distinguished fragment chromosomes seemed to pair rather con-



FIGS. 1–14.— Stages of first meiotic division of pollen mother cell. Fig. 1, nucleolus-satellite relation at early diakinesis in normal ($2n = 14$). Fig. 2, diakinesis in $P_{II}L_{1-22}$ ($2n = 13 + f$). Fig. 3, diakinesis in $P_{II}J_{1-2}$ ($2n = 14 + f$). Figs. 4, 5, anaphase figures of $P_{II}L_{30-9}$ (inversion heterozygote). Fig. 6, diakinesis in $P_{II}L_{30-12}$ (translocation heterozygote). Note nucleolus-satellite relation and separation of satellites from main body of chromosome. Figs. 7–9, $P_{II}N_{9-1}$ ($2n = 13$); fig. 7, diakinesis; figs. 8, 9, telophase. Figs. 10–14, $P_{II}L_{28-14}$ ($2n = 13$); figs. 10, 11, diakinesis; figs. 12, 13, metaphase; fig. 14, telophase figures.

sistently with their normal partners (fig. 2), although the frequency of chiasmata was reduced—as might be expected.

Meiotic studies revealed one inversion and several reciprocal translocations. The single inversion must have been rather large, since 56 per cent of 147 figures examined in anaphase of the first division showed an inversion bridge with accompanying fragment. Nothing unusual was seen in the meiotic behavior of the translocations. One was found to involve the satellited chromosome; another (fig. 6) was characterized by a very high frequency of quadrivalent formation. All of the foregoing types of aberration have been recovered frequently from X-rayed plant material (9).

Monosomics may also be induced by X-ray treatment, but this treatment has not ordinarily been considered a technique for producing them, especially in diploid plants. It is assumed that in the ordinary sense *Petunia* is a diploid, its haploid number, seven, being the lowest yet found in the Solanaceae. Considerable attention was therefore paid to three monosomics which appeared in the X_1 generation.

One of these, plant $P_{II}N9$–1, appeared in a family which could be traced to pollen treated with 30,000 r. Earlier observations on the root tips of this plant had revealed the presence of a chromosome fragment in addition to thirteen normal chromosomes. This observation was verified by four separate collections of root tips. On the other hand, three preparations of pollen mother cells from this plant showed only thirteen chromosomes, none of which appeared abnormally short. As a matter of fact, the chimeral condition of this plant was not realized until long after the growing season. The preserved material was limited but was sufficient to establish the pairing relations in its thirteen chromosomes.

Figure 7 illustrates a typical diakinesis figure of this monosomic. The odd chromosome remained unpaired in by far the greater number of figures (87 per cent of twenty-four analyzable ones). In fact, its attachment with a rod bivalent in three figures could not be interpreted as the effect of chiasmata with any degree of certainty. At anaphase of the first division the odd chromosome tended to lag on the equatorial plate (figs. 8, 9). This lagging occurred in 71 per cent of the thirty-eight telophase figures examined. Flowers of this plant set seed by selfing, but no studies have yet been made of its progeny.

Another monosomic, $P_{II}N9$–9, was recovered from the same population. No meiotic studies could be made, since this plant died before flowering.

The remaining monosomic, $P_{II}L28$–14, was a product of pollen receiving 25,000 r. In this case some fifteen separate observations on root tips and pollen mother cells affirmed the count of thirteen chromosomes. This plant as a seedling had deformed leaves, but in later development it produced small leaves of normal shape. Flowers were considerably smaller than the smallest segregates in the F_2 of the original cross. In fact, the whole plant could be well described as diminutive. It was nevertheless healthy and required no special cultural care.

Satisfactory preparations of meiotic divisions were difficult to obtain in the case of this monosomic. Those which could be relied upon showed a behavior of the odd chromosome quite different from that of the previous case. In fifteen out of twenty-five clearly defined diakinesis figures the odd chromosome was associated in a trivalent configuration. Although this is a relatively small num-

ber, the pairing association was also observed in the majority of figures in other preparations. Typical meiotic behavior is demonstrated in figures 10–14. Since it has not been possible to distinguish morphologically between the seven *Petunia* bivalents at diakinesis, it was uncertain whether the odd chromosome was always associated with the same bivalent, yet it was never attached to the satellited bivalent. Furthermore, the behavior is so remarkably regular for a monosomic that it is doubtful whether more than one bivalent was concerned.

This high frequency of pairing was reflected in the more regular performance of the odd chromosome at anaphase. It lagged behind the other chromosomes in no more than 2 per cent of a great number of figures. This estimate was confirmed by the frequency of tetrad microspore groups which included micronuclei.

The unusual pairing behavior of this odd chromosome is attributed to genetic homology between it and the chromosomes to which it is so strongly attracted —according to the accepted explanation of chromosome pairing. As to the origin of the homologous section of this chromosome, two possibilities exist: either it is the result of a translocation effected by the X-rays or it is a duplication normally present in the haploid complement of *Petunia*. These alternatives are diagrammed in figure 15.

In the case of a naturally occurring duplication, the X-ray treatment would in some way have prevented the chromosome bearing this duplication (fig. 15 *du*) from migrating at anaphase to the sperm nucleus which functioned in fertilizing the egg nucleus. An inactivation of the centromere by an effective ionization might account for this misbehavior. Other possible ways in which this effect might have been produced are suggested in the study by SWANSON (22) of X-ray effects on chromosome behavior in the division of the generative nucleus in pollen tubes of *Tradescantia*. The "odd" chromosome (the one bearing the duplication) would thus enter the zygote of the monosomic from the female gamete. It would pair as an end member of the trivalent in meiosis of the monosomic.

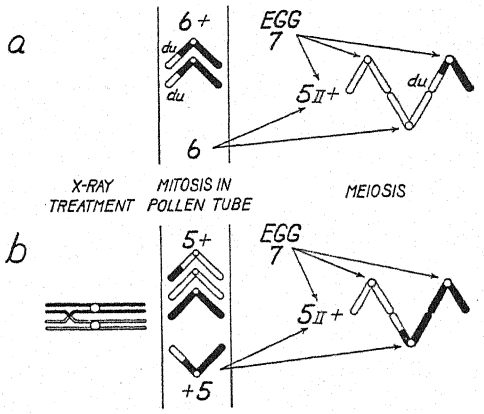


FIG. 15.—Diagrams of hypothetical origins of odd chromosome in monosomic, $P_{II}L28$–14: *a*, case of naturally occurring duplication (*du*, segment which is duplicated); *b*, case of X-ray-induced duplication.

If the homologous section could be related to the X-ray treatment, as illustrated in alternative *b* of figure 15, the radiation would be required to account for the lagging of chromatids as well as a translocation between one of the lagging chromatids and another chromatid of the six remaining chromosomes. The spreading effect of a single ionization might be held responsible if all the regions concerned were sufficiently close at the time of treatment, but it is certainly much more likely that two independent ionizations were involved. According to this alternative the chromosome with a duplication would be contributed by the sperm and in the monosomic it would

pair as the middle member of the trivalent.

In the lack of conclusive evidence, neither hypothesis can be proved or disproved; however, two points might merit attention at this point. First is the double task assigned to X-rays in the hypothesis that the radiation is responsible for the homologous section of the odd chromosome. The probability of such an occurrence is remote in view of the relative frequency of induced aberration observed in this experiment. Thus at the dose of X-rays which produced this deficient gamete, only 11 per cent of the X_1 generation showed gross chromosomal aberrations. Furthermore, in only two cases were plants found which showed two independent aberrations. In the light of these facts it does seem rather unlikely that two independent ionizations would have affected the same chromosome. Both effects might be attributed to one ionization, but this is also a rather remote possibility.

In regard to the explanation based on a naturally occurring duplication, it may seem somewhat extraordinary to postulate internal polyploidy in the complement of a plant which has the lowest chromosome count of a relatively well explored family, yet this same condition has been demonstrated by CATCHESIDE (3) in an *Oenothera* haploid. Then, too, evidence from other sources suggests the existence of duplicated regions in the *Petunia* complement. In the progeny of triploids and trisomics in *Petunia*, LEVAN (15) and, more recently, the writer have observed the presence of fragment chromosomes. Possibly these shortened chromosomes are the products of pairing in an allosyndetic sense. If internal duplications exist, competition for pairing might lead to pairing of what are ordinarily considered non-homologous chromosomes. It is conceivable that chromosomes thus paired might be displaced with respect to their ends and centromeres, so that crossovers in the paired region would lead to shortened chromosomes (fragment chromosomes), lengthened chromosomes, and possibly even dicentric and acentric chromosomes.

Other phenomena which indicate internal polyploidy are known in *Petunia*. According to unpublished findings of the writer, the effects of chromosomal unbalance on phenotype, viability, and fertility are lacking to a surprising extent. Here, and in the work of LEVAN (16), seedlings from triploid by diploid crosses exhibited chromosome numbers ranging continuously from fourteen to twenty. Even the most unbalanced of these were fairly vigorous, deviated little from the diploid in phenotype, and yielded considerable viable seed. Such results could hardly be expected in a plant which is diploid in the strict sense. Furthermore, several workers have experienced relative difficulty in obtaining cases of well-defined disomic Mendelian segregation in *Petunia*. Internal duplications and the consequent presence of genes in quadruplicate could also account for these findings. The tolerance of *Petunia* pollen to relatively high X-ray doses might also be an indication of internal polyploidy.

Neither of these points per se constitutes proof, but together they do intimate that the more likely explanation of the unusual pairing in this monosomic is to be found in naturally occurring duplications.

Summary

1. Pollen of diploid *Petunia* was subjected to X-ray doses varying from 10,000 to 100,000 r and was subsequently applied to stigmas of untreated plants. Pollen which had been exposed to doses

as high as 50,000 r functioned to produce viable seed. The half-kill dose for the number of plants produced per flowers pollinated was approximately 9,000 r.

2. Techniques were applied to the X_1 and X_2 generations in order to reveal reduced pseudogamy or pseudogamous development of diploid individuals from eggs in which a doubling of the chromosome number had occurred. No evidence of either phenomenon was observed.

3. Thirty-three plants produced by X-rayed pollen were found to have gross chromosomal deviations, including inversion, translocation, duplication, and deficiency. One-third were chimeras in which the chromosome condition of pollen mother cells differed, usually in the presence or absence of a fragment chromosome, from that of the roots. Loss of the fragment in the earliest division of the embryo presumably accounts for these differences.

4. Two monosomics were obtained. In one the odd chromosome nearly always appeared as a univalent, which usually failed to be included in either telophase nucleus in the first division and appeared as a micronucleus in later stages. In the other the odd chromosome was associated in a trivalent in the majority of figures and never lagged at anaphase of the first or second divisions. This unusual pairing is attributed either to a duplication present in the normal complement of *Petunia* or to a translocation induced by the X-ray treatment. Reasons are presented which suggest that the former hypothesis is the more likely one.

The writer gratefully acknowledges the guidance of the late Dr. E. M. EAST in the early work on this problem. Acknowledgment is also made to the Huntingdon Memorial Hospital of Boston for the use of X-ray equipment, and to Dr. MARGARET FERGUSON, who kindly furnished seed of inbred and hybrid lines of *Petunia*.

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LITERATURE CITED

1. BHADURI, P. N., Studies on the embryogeny of the Solanaceae. I. Bot. Gaz. 98:283–295. 1936.
2. BLAKESLEE, A. F., The present and potential service of chemistry to plant breeding. Amer. Jour. Bot. 26:163–171. 1939.
3. CATCHESIDE, D. G., The chromosomes of a new haploid *Oenothera*. Cytologia 4:68–113. 1932.
4. CHRISTOFF, M., and PAPASAVA, G., Sur les causes de l'augmentation du nombre chromosomique des cellules somatiques de certains Pétunias. La Cellule 45:353–356. 1937.
5. DARLINGTON, C. D., Polyploidy, crossing-over, and heterochromatin in *Paris*. Ann. Bot. n.s. 5:203–216. 1941.
6. EAST, E. M., The production of homozygotes through induced parthenogenesis. Science 72: 148–149. 1930.
7. ———, The origin of plants of maternal type which occur in connection with interspecific hybridizations. Proc. Nat. Acad. Sci. 16:377–380. 1930.
8. FERGUSON, MARGARET C., *Petunia*. Proc. 6th Internat. Cong. Genet. 2:327–328. 1932.
9. GOODSPEED, T. H., and UBER, F. M., Radiation and plant cytogenetics. Bot. Rev. 5:1–48. 1939.
10. IVANOV, M. A., Experimental production of haploids in *Nicotiana rustica* L. Genetica 20: 295–397. 1938.
11. JANAKI-AMMAL, E. K., Chromosome diminution in a plant. Nature 146:839–840. 1940.
12. JØRGENSEN, C. A., The experimental formation of heteroploid plants in the genus *Solanum*. Jour. Genet. 19:133–210. 1938.

13. KATAYAMA, Y., Haploid formation by X-rays in *Triticum monococcum*. Cytologia 5:235–237. 1934.
14. KIHARA, H., Formation of haploids by means of delayed pollination in *Triticum monococcum*. Bot. Mag. Tokyo 54:178–185. 1940.
15. LEVAN, A., On chromosome fragments in *Petunia* Svensk Bot. Tids. 31:1–10. 1937.
16. ———, Chromosome numbers in *Petunia*. Hereditas 23:99–112. 1937.
17. MCCLINTOCK, BARBARA, A 2n–1 chromosomal chimera in maize. Jour. Hered. 20:218. 1929.
18. RANDOLPH, L. F., Card mounts for handling root tips in the paraffin method. Stain Technol. 15:45–48. 1940.
19. REDINGER, K., Über die Entstehung diploider Embryonen aus unbefruchteten mit gattungsfremden Pollen bestäubten Samenanlagen von *Petunia nyctaginiflora*. Biol. Zentralbl. 58:142–151. 1938.
20. RICK, C. M., Cytological irregularities induced in *Petunia* by X-ray treatment of pollen. Genetics 27:164. 1942.
21. STEBBINS, G. L., JR., Apomixis in the angiosperms. Bot. Rev. 7:507–542. 1941.
22. SWANSON, C. P., A comparison of chromosomal aberrations induced by X-ray and ultra-violet radiation. Proc. Nat. Acad. Sci. 26:366–373. 1940.
23. VAN OVERBEEK, J., CONKLIN, MARIE E., and BLAKESLEE, A. F., Chemical stimulation of ovule development and its possible relation to parthenogenesis. Amer. Jour. Bot. 28:647–655. 1941.
24. WEBBER, J. M., Polyembryony. Bot. Rev. 6:575–598. 1940.

ANALYSIS OF VARIANCE AND COVARIANCE OF CHROMOSOMAL ASSOCIATION AND BEHAVIOR DURING MEIOSIS IN CLONES OF DACTYLIS GLOMERATA[1]

W. M. MYERS[2]

Introduction

In general, autopolyploids are irregular in meiosis, the irregularities resulting in decreased fertility and in the production of aneuploid progeny. It has been shown by MÜNTZING (10, 12) and MYERS and HILL (16, 17) that *Dactylis glomerata* L., which behaves cytogenetically as an autotetraploid (x = 7), is not an exception to the general rule. The latter workers (17) found statistically significant differences among plants of this species in frequency of quadrivalents at diakinesis, unpaired chromosomes at metaphase I, lagging and dividing chromosomes at anaphase I, and micronuclei in the quartets. The occurrence of differences among plants in degree of meiotic irregularity suggests the possibility of producing strains with greater regularity of meiosis than the average of the species. Such strains should, consequently, be more stable in chromosome number and more fertile than unselected material. For an evaluation of this possibility, more knowledge is required regarding the extent and nature of variation in the various features of meiosis which have been investigated.

Material and methods

Nine clones of *D. glomerata* were selected as representative of the range in degree of meiotic irregularity found among the twenty studied previously by MYERS and HILL (17). These clones were planted in the field in rows distributed at random in three replications. Microsporocyte material was collected and fixed from one plant in each replication of six clones in 1940 and from all nine clones in 1941. The material was fixed in acetic-alcohol and stored at low temperature in the fixing solution until studied. All data were collected from fresh aceto-carmine smear slides.

For the analysis of variance involving data for 2 years, the procedure recommended by COCHRAN (1) for experiments with perennial crops has been used. For the analysis of covariance, SNEDECOR'S (22) outline was followed.

Experimental results

DIAKINESIS.—For each replication of the clones, data on quadrivalent frequency were collected from an average of 38 diakinesis sporocytes in 1940 and 24 sporocytes in 1941. In 1941, the chiasma frequency, expressed as half-chiasmata per chromosome, was determined from the same diakinesis sporocytes. These data are summarized in table 1, with the six clones arranged in ascending order on the basis of average number of quadrivalents per sporocyte for the 2 years. The averages ranged from 3.1 to 4.2 for the six clones.

The mean numbers of quadrivalents found in the three plants studied only in 1941 were within the range of the six plants studied in both years. In addition to quadrivalents and bivalents, there were found at diakinesis in these plants some sporocytes with univalents and others with a univalent plus a trivalent. The frequency of the latter type was low, the number of trivalents per sporocyte

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varying from 0 to 0.09 (2 trivalents in 23 sporocytes) among the replicates of the nine clones. Therefore, combining the trivalents with the quadrivalents to give multivalent frequency would not change significantly the relationship among plants that is shown by the average number of quadrivalents.

quency in 1941 as in 1940, while the means for the remaining three clones showed greater differences between years.

The low mean square for replications suggested that this character was not greatly affected by the soil variations involved in this experiment. This is indicated further by a comparison of the mean

TABLE 1

FREQUENCY OF HALF-CHIASMATA PER CHROMOSOME AND QUADRIVALENTS PER SPOROCYTE AT DIAKINESIS IN NINE CLONES OF DACTYLIS GLOMERATA AND INTRA-PLANT CORRELATIONS OF CHIASMA FREQUENCY WITH QUADRIVALENT FREQUENCY IN 1941

Clone no.	Half-chiasmata per chromosome in 1941 in				Quadrivalents per sporocyte in			r*	D/F within plants
	II	IV	Difference	Total	1940	1941	Average		
OG 6(12).....	1.60	1.84	0.24	1.69	3.0	3.2	3.1	+0.559†	72
OG 48(28).....	1.82	1.90	0.08	1.86	3.9	3.0	3.4	+0.126	73
OG 7(20).....	1.64	1.86	0.22	1.73	4.0	3.5	3.8	+0.484†	44
OG 42(5).....	1.87	1.92	0.05	1.90	3.8	4.3	4.0	+0.113	57
OG 48(92).....	1.74	1.89	0.15	1.82	4.1	4.0	4.0	+0.308†	87
OG 2(11).....	1.80	1.91	0.11	1.87	4.1	4.2	4.2	+0.312†	97
OG 48(48).....	1.71	1.86	0.15	1.77	.....	3.0	.....	+0.304†	70
OG 48(47).....	1.75	1.91	0.16	1.82	.....	3.1	.....	+0.228	72
OG 2(3).....	1.71	1.85	0.14	1.78	.....	3.5	.....	+0.470†	53

* Correlation coefficient between total number of half-chiasmata per chromosome and number of quadrivalents per sporocyte among sporocytes within plants of clones in 1941.

† Exceeds value of r for P of 0.01 according to FISHER'S (5) table VA.

The analysis of variance of quadrivalent frequency in the six clones with data for 2 years (table 2) showed that the variances for clones and for the interaction of clones × years were statistically significant. The significant interaction of clones × years indicates that some factor or factors of the environment had a measurable effect upon quadrivalent frequency and that the effects of those factors were not the same for the different clones. The variance for clones was not significantly greater than the variance for clones × years, and the variance for years was not significantly greater than that for error (b). From table 1 it may be seen that three of the clones, that is, OG 6(12), OG 48(92), and OG 2(11), had nearly the same quadrivalent frequency in 1941 as in 1940, while the means for the remaining three clones showed greater differences between years.

square for error with the mean square within plants (that is, within replicates of each clone) obtained in 1941 (table 3). As pointed out by IMMER (7), MYERS (14), and others, the error mean square is composed of the mean sampling variance (in this case the variance of the mean within replicates) plus the true variance between clones within replications. In this experiment the data on quadrivalent frequency per plant were based on an average of 24.48 sporocytes in 1941. Therefore the mean variance within plants was $1.6516 \div 24.48 = 0.0675$. Actually this value is slightly higher than the error mean square of 0.0416 (the estimated true variance between clonal rows within replications is a negative quantity), indicating that the true variance must have

been very low. Since this variance is nearly zero, it would seem appropriate to accept variance within plants as a fairly reliable estimate of error for comparing different plants or clones with regard to quadrivalent frequency.

The number of half-chiasmata per chromosome (table 1) ranged from 1.69 to 1.90 for the nine clones in 1941. Data on this character were not collected in 1940. An analysis of variance (table 3) indicated that the differences among clones were highly significant. The uncontrolled variation in this character was low; the standard error of a single determination (average of 24 sporocytes per replication) was only 0.03 (table 3). Furthermore, the mean square for replications was lower than that for error, indicating little or no effect of soil heterogeneity upon chiasma frequency in this experiment. This conclusion is supported by the comparison of the error mean square with the variance within plants. The variance of the mean within plants was 0.0128 ÷ 24.48 = 0.0005. Since the error mean square was 0.0009, the estimated true variance between clonal rows was only 0.0004. The value of F for comparing the error mean square with the

TABLE 2

ANALYSES OF VARIANCE OF FEATURES OF CHROMOSOMAL ASSOCIATION AND BEHAVIOR DURING MEIOSIS IN SIX CLONES OF DACTYLIS GLOMERATA FROM WHICH DATA WERE OBTAINED FOR 1940 AND 1941

Variance due to	D/F	No. of quadrivalents		Percentage of sporocytes				Percentage of quartets with micronuclei	
				With univalents at MI		With laggards at AI			
		Mean square	F	Mean square	F	Mean square	F	Mean square	F
Clones	5	0.9466	18.94†	227.	23.16†	909.	18.92†	1364.	34.34†
Replications	2	0.0448	.....	112.	11.44†	20.	.....	76.	1.91
Error (a)	10	0.0500	.....	10.	.....	48.	.....	40.	.....
Years	1	0.1202	3.02	206.	32.41†	8.	.....	511.	22.79†
Clones×years	5	0.3412	8.58†	78.	12.33†	52.	5.54*	203.	9.08†
Replications×years	2	0.0607	.....	21.	3.26	2.	.....	126.	5.63*
Error (b)	10	0.0398	.....	6.	.....	9.	.....	22.	.....

* Exceeds F for P of 0.05.
† Exceeds F for P of 0.01.

TABLE 3

ANALYSIS OF VARIANCE BETWEEN AND WITHIN PLANTS OF FREQUENCY OF HALF-CHIASMATA PER CHROMOSOME AND OF QUADRIVALENTS PER SPOROCYTE IN NINE CLONES OF DACTYLIS GLOMERATA IN 1941

Variance due to	Half-chiasmata per chromosome			Quadrivalents per sporocyte		
	D/F	Mean square	F	D/F	Mean square	F
Clones	8	0.0141	15.22*	8	0.7464	17.95*
Replications	2	0.0002	.....	2	0.0146	.....
Error	16	0.0009	.....	16	0.0416	.....
Within plants† of clones	634	0.0128	.....	634	1.6516	.....

* Exceeds F for P of 0.01.

† Calculated by averaging mean squares for individual plants of each clone. When calculated by pooling sums of squares and degrees of freedom for within plants, the mean squares were 0.0125 and 1.6427, respectively. The slight discrepancy arose from the variable numbers of sporocytes recorded for the different clones.

variance of the mean within plants was 1.800, which gives P of less than 0.05, suggesting that variance within plants may not furnish a reliable estimate of error for comparing different plants.

The data on chiasma frequency were recorded separately for the chromosomes associated as bivalents and as quadrivalents (table 1). In all clones the number of half-chiasmata per chromosome was higher in the chromosomes of the quadrivalents than in those of the bivalents, the differences ranging from 0.05 to 0.24 among the nine clones. The results suggest that within sporocytes chiasma frequency was a limiting factor in the formation of quadrivalents, at least in some of the clones. The differences were greatest in those clones with a low chiasma frequency and least in the clones with a high chiasma frequency. This relationship would be expected on the assumption that the differences resulted from the limitation of chiasmata number upon quadrivalent formation.

To test the importance of chiasma frequency in determining variations in quadrivalent frequency among sporocytes within plants of clones, correlation coefficients were calculated. To obtain the correlation within plants (replicates) of the same clones, the sums of products and the mean squares were calculated separately for each plant, the values for the three plants of the clone were added, and r was determined from the pooled data. Of the nine correlation coefficients (table 1) three were not significant, whereas six gave P values of less than 0.01. In all three of the clones with nonsignificant coefficients, the differences in half-chiasmata between the chromosomes of the bivalents and of the quadrivalents were among the lowest obtained. In all clones the correlation coefficients were relatively low, the highest value being 0.559. In other words, not more than 31 per cent of the squared variation in quadrivalent frequency among sporocytes within plants can be attributed to variation in numbers of half-chiasmata. The results suggest that other factors, such as chance location of the chiasmata, variation among sporocytes in the relative distances between homologues at the time of initiation of prophase, and timing balance during prophase play important roles in conditioning differences among sporocytes in numbers of quadrivalents.

UNIVALENTS AT METAPHASE I AND LAGGING AND DIVIDING CHROMOSOMES AT ANAPHASE I.—An average of 160 metaphase I sporocytes from each replication of each clone in 1940 and of 182 sporocytes in 1941 were examined. Three of the clones, that is, OG 2(3), OG 6(12), and OG 7(20), were high in percentage of sporocytes with one or more univalents (table 4). On the other hand, the amount of asynapsis found in OG 48(28) and OG 48(47) was low; in fact, the averages for these clones were within the range found among normal plants of diploid *Lolium perenne* (13). The remaining four plants ranged between these extremes. In the analysis of variance of the data on percentage of sporocytes with univalents (table 2) the mean squares for clones and for replications were significantly larger than that for error (a), while the mean squares for years and for the interaction of clones × years significantly exceeded mean square for error (b). Apparently this character is affected both by environmental fluctuations from year to year and by heterogeneity in the field. Some question may be raised regarding the legitimacy of using the analysis of variance for percentage data covering the range found in these experiments. In this case, however, the magnitude of the differences was so great as to leave

little question of their statistical significance. The rank of the clones in number of univalents per 100 sporocytes was the same as in percentage of sporocytes with univalents. The analysis of variance of these data yielded results very similar to those obtained for percentage of sporocytes with univalents.

parison of mean square for the interaction of clones × years with mean square for error (b), F was greater than F for P of 0.05. Since percentage data covering a considerable range were involved in the analysis, this difference may be of doubtful significance. The differences among replications and between years were not

TABLE 4

PERCENTAGE OF SPOROCYTES WITH UNIVALENTS AT METAPHASE I, WITH LAGGING AND DIVIDING CHROMOSOMES AT ANAPHASE I, AND WITH MICRONUCLEI IN THE QUARTETS IN NINE CLONES OF DACTYLIS GLOMERATA

Clone no.	Percentage of sporocytes								
	With univalents at MI in			With laggards at AI in			With micronuclei in the quartets		
	1940	1941	Average	1940	1941	Average	1940	1941	Average
OG 6(12)	12	29	20	36	36	36	32	56	44
OG 48(28)	3	3	3	4	2	3	4	3	4
OG 7(20)	14	9	12	28	19	24	35	26	30
OG 42(5)	4	9	6	8	13	10	11	18	14
OG 48(92)	6	11	8	8	16	12	6	13	10
OG 2(11)	4	9	6	5	9	7	5	21	13
OG 48(48)	.....	5	.....	.....	7	.....	.....	4	.....
OG 48(47)	.....	3	.....	.....	2	.....	.....	2	.....
OG 2(3)	.....	24	.....	.....	29	.....	.....	28	.....

The data for each clone on frequency of lagging and dividing univalents at anaphase I were obtained from an average of 108 sporocytes per replication in 1940 and of 95 sporocytes in 1941. The clones occupied approximately the same order with regard to percentage of anaphase I sporocytes with laggards (table 4) and number of laggards per 100 sporocytes as they did with regard to metaphase I univalents. Also in the frequency of laggards at anaphase I, the two clones OG 48(28) and OG 48(47) were only slightly less regular than the average of the normal plants of *Lolium perenne* (13).

In the analysis of variance of percentage of anaphase I sporocytes, the results indicated highly significant differences among plants (table 2). Also, for com-

significant. The mean squares for errors (a) and (b) were higher in the analysis of variance of anaphase I laggards than of metaphase I univalents, although the means of these characters were not greatly different. It is possible that the greater random variation found in anaphase I laggards may have arisen from the difficulty of determining accurately in certain instances whether chromosomes were lagging and starting to divide equationally. The analysis of variance of number of laggards per 100 anaphase I sporocytes likewise showed highly significant differences among plants. In this analysis, the value of P for comparing mean square for the interaction of clones × years with mean square for error (b) was considerably less than 0.01, indicat-

ing that the interaction was statistically significant.

DICENTRIC CHROMATID BRIDGES AND ACENTRIC FRAGMENTS AT ANAPHASE I.—Dicentric chromatid bridges and acentric fragments were observed in some anaphase I sporocytes in each of the nine clones. For the six clones with data for 2 years, sporocytes with bridges and fragments ranged from 2 to 8 per cent. These differences were found to be statistically significant, suggesting that inversions of different lengths occurred in the six clones or that there were more inverted segments in some clones than in others. There was no reason to suppose that such differences would not be found. Among the twenty plants studied by MYERS and HILL (17), chromatid bridges and acentric fragments were found in all but two. One of these was OG 6(12), which showed an average of 6 per cent of the anaphase I sporocytes with such configurations in the present study. Therefore, at least nineteen of the twenty clones studied are probably heterozygous for one or more inversions.

DISJUNCTION AT ANAPHASE I.—While examining the anaphase I sporocytes for laggards, the number of chromosomes moving to each pole was recorded for all sporocytes in which the chromosomes were so well spread that both groups could be counted accurately. Of the 520 sporocytes recorded, 507 had a 14–14 distribution and 13 (2.5 per cent) had a 13–15 distribution. In many other sporocytes in which only one group of chromosomes could be counted the number was almost always 14.

QUARTETS WITH MICRONUCLEI.—For determining the frequency of quartets with micronuclei, an average of 180 and 169 sporocytes per replication were examined from each clone in 1940 and 1941, respectively. Three of the clones, OG 48 (28), OG 48(47), and OG 48(48), had only 4, 2, and 4 per cent, respectively, of the sporocytes with micronuclei. Two of these, OG 48(28) and OG 48(47), were also low in incidence of unpaired chromosomes at metaphase I and laggards at anaphase I. In the analysis of variance of these data (table 2), the mean squares for clones, years, and clones × years were significantly greater (P was less than 0.01) than their appropriate error mean squares.

Quartets with two or more micronuclei occurred frequently, particularly in those clones in which a high percentage of the quartets had micronuclei. As a result, the range among clones in total number of chromosomes lost per 100 sporocytes (as measured by the number of micronuclei in the quartets) was greater than indicated by the range in percentage of quartets with micronuclei. In this regard also the clones OG 48(28), OG 48(47), and OG 48(48) were comparatively regular.

COVARIANCE OF BEHAVIORS AT DIFFERENT STAGES OF MEIOSIS.—For the analysis of covariance, replicated data collected in 1941 from the nine clones were used throughout. A summary of the analysis of covariance of half-chiasmata per chromosome and quadrivalent frequency per sporocyte is presented in table 5. The significance of covariance cannot be tested directly; instead the correlation (r) or regression (b) coefficients may be calculated (table 6) and the significance of either one tested. If one is significant, the other also is significant, and vice versa. In this paper the significance of r has been determined from FISHER'S table VA (5). For the correlation of chiasma with quadrivalent frequency, r for clones was not significant but r for total exceeded that necessary for P of 0.01.

The remaining correlation coefficients (table 6) were calculated from the analysis of covariance in the same manner. In all analyses the mean squares for clones significantly exceeded mean square for error (P was less than 0.01 in all cases).

TABLE 5

ANALYSIS OF COVARIANCE OF HALF-CHIASMATA PER CHROMOSOME (x) AND NUMBER OF QUADRIVALENTS PER SPOROCYTE (y) USING DATA OBTAINED FROM NINE CLONES OF DACTYLIS GLOMERATA IN 1941

Variance due to	D/F	Sums of squares due to			Errors of estimate			
		Sx^2	S(xy)	Sy^2	Sums of squares	D/F	Mean square	F
Replications	2	0.0004	−0.0017	0.0291	.....	.....	.....	.....
Clones	8	0.1129	0.4248	5.9710	.....	.....	.....	.....
Error	16	0.0148	0.0040	0.6652	0.6651	15	0.0443	.....
Clones+error	24	0.1277	0.4288	6.6362	5.1964	23	.....	.....
Difference for testing adjusted clone means					4.5313	8	0.5664	12.77*

* Exceeds F for P of 0.01.

TABLE 6

CORRELATION COEFFICIENTS CALCULATED FROM INDICATED ANALYSES OF COVARIANCE

Variance due to	D/F	A with B*	A with C*	B with C*	B with D*	C with D*	D with E*
		r	r	r	r	r	r
Clones	7	0.517	−0.601†	0.053	0.078	0.969‡	0.928‡
Error	15	0.041	−0.037	−0.192	−0.252	0.363	0.176
Total	25	0.462‡	−0.576‡	0.028	0.026	0.861‡	0.758‡

* A, half-chiasmata per chromosome; B, number of quadrivalents per sporocyte; C, percentage of metaphase I sporocytes showing univalents; D, percentage of anaphase I sporocytes showing laggards; E, percentage of quartets showing micronuclei.

† Exceeds r for P of 0.10 (table VA, 5).

‡ Exceeds r for P of 0.01 (table VA, 5).

A significant negative correlation coefficient (and therefore also regression coefficient) was found between chiasma frequency and percentage of metaphase I sporocytes with univalents. Significant positive correlation coefficients occurred between metaphase I with univalents and anaphase I with laggards, and between anaphase I with laggards and quartets with micronuclei. From present knowledge of chromosome pairing and behavior during meiosis, these relationships were expected. The correlation coefficients of quadrivalent frequency with metaphase I univalents and with anaphase I laggards were very small and not significantly different from zero. These results agree with the previous report by MYERS and HILL (17) of absence of correlation between these characters.

Just as important as the correlation between the various characters is the question whether there are significant differences among clones in one character

which cannot be accounted for by its regression upon another. This problem can be investigated by use of regression, calculating estimated values for the dependent variable on the basis of its regression on the independent variable and subjecting the estimated value to the analysis of variance. A more direct approach to the problem by calculation of errors of estimate from the covariance analysis has been given by SNEDECOR (22). The latter method was used in these investigations. In the analysis of covariance of chiasma frequency with quadrivalent frequency (table 5), it can be seen under errors of estimate that the mean square of the difference for testing the adjusted clone means was significantly greater than the error mean square (F was greater than that for P of 0.01). The results show that there were statistically significant differences among clones in quadrivalent frequency which could not be accounted for by differences in chiasma frequency.

Errors of estimate were calculated in a similar manner from each analysis of covariance and are summarized in table 7. In all but one case the differences for testing adjusted clone means were significantly greater than error, indicating that there were real differences among clones in each dependent variable which could not be accounted for by its regression on the independent variable. The single exception was in the covariance of percentage of metaphase I sporocytes having univalents with the percentage of anaphase I sporocytes having laggards. This indicates that in this investigation all significant differences among clones in frequency of laggards at anaphase I may be attributed to variations in incidence of univalents at metaphase I.

TABLE 7

ERRORS OF ESTIMATE CALCULATED FROM ANALYSIS OF COVARIANCE OF VARIOUS CHARACTERISTICS OF MEIOSIS IN NINE CLONES OF DACTYLIS GLOMERATA

VARIANCE DUE TO	D/F	A WITH C*		B WITH C*		B WITH D*		C WITH D*		D WITH E*	
		Mean square	F	Mean square	F	Mean square	F	Mean square	F	Mean square	F
Error.....	15	12.58	.....	9.20	.....	24.62	.....	58.41	....	36.06	.....
Difference for testing adjusted clone means.....	8	160.38	12.75†	257.83	28.02†	493.83	20.06†	30.55	....	803.35	22.28†

* A, half-chiasmata per chromosome; B, number of quadrivalents per sporocyte; C, percentage of metaphase I sporocytes showing univalents; D, percentage of anaphase I sporocytes showing laggards; E, percentage of quartets showing micronuclei.
† Exceeds r for P of 0.01 (table VA, 5).

Discussion

From the standpoint of plant breeding, the significant differences in meiotic irregularity found among clones of *Dactylis glomerata* are of considerable importance, since they suggest the possibility of selecting strains with greater meiotic regularity and probably also greater fertility. Although, as MÜNTZING (11) and RANDOLPH (20) have suggested, physiological factors may be the chief cause of sterility in some autopolyploids, there seems to be little question but that meiotic irregularity also tends to decrease fertility. At least two of the nine plants re-

ported in this paper were very nearly as regular in meiosis as normally occurring plants of the diploid species *Lolium perenne* (13). Since the differences among plants in this investigation were maintained in different replications and through two seasons, it seems logical to conclude that they are heritable. Progeny tests, the results of which will be presented elsewhere, have confirmed this supposition.

There have been reported in the literature numerous examples of genetically controlled abnormalities of meiosis, especially asynapsis at diakinesis and metaphase I, but relatively few cases have been published of small heritable variations in meiotic behavior among apparently normal plants of a species. Variations in chiasma frequency among plants or clones of the same species have been reported by DARLINGTON (3) for *Fritillaria imperialis;* SAX (21) for *Secale cereale, Vicia faba,* and *Tradescantia* species; KAKHIDZE (8) for *Secale cereale;* and MYERS (13) for diploid *Lolium perenne.* In autopolyploid *Lycopersicum esculentum,* JORGENSEN, LESLEY and LESLEY, and UPCOTT (see UPCOTT, 23, for literature citations) reported different quadrivalent frequencies. GILES (6) found fewer quadrivalents in autotetraploid *Tradescantia* than had been reported previously by ANDERSON and SAX and by DARLINGTON. He (6) attributed the difference to lower chiasma frequency or to a greater degree of chromosomal differentiation. Since the data reported for *Lycopersicum* and *Tradescantia* were not collected under the same conditions, it is impossible to judge the significance of the differences obtained. Variations in quadrivalent frequency among plants of autopolyploid grasses have been found by MYERS and HILL (16, 17) for *Agropyron cristatum, Arrhenatherum elatius,* and *Dactylis glomerata* and by MYERS (15, and unpublished) for hexaploid *Phleum pratense* and induced autotetraploid *Lolium perenne.*

Less attention has been given to variations in chromosome behavior and loss in later stages of meiosis. MYERS and POWERS (18) found heritable differences in chromosome loss among plants of *Triticum aestivum,* and MYERS (13) reported variations among plants of diploid *Lolium perenne* in incidence of unpaired chromosomes at metaphase I, laggards at anaphase I, and micronuclei in the quartets. Differences in these characters also were found among plants of the polyploid grass species studied by MYERS and HILL (16, 17) and MYERS (15). The results suggest that material is available in many autopolyploids, both natural and induced, for the selection of types with greater meiotic regularity.

With this possibility in mind, there logically follows the question of which feature of meiotic irregularity is of greatest importance in conditioning instability and decreased fertility. DARLINGTON (4), KOSTOFF (9), and others have attributed the meiotic chromosomal irregularities of autopolyploids to the multivalent association of chromosomes during synapsis. As a result of this assumption there has been a tendency to judge the regularity of autopolyploids on the basis of frequency of multivalents. Although the results with *Dactylis glomerata* do not contradict the hypothesis of the basic importance of multivalent association in conditioning meiotic irregularity, they do emphasize that multivalent frequency at diakinesis and metaphase I is an inadequate criterion of the amount of irregularity during meiosis. In these investigations frequency of quadrivalents was correlated neither with frequency of asynapsis at metaphase I nor with frequency of lagging at anaphase I. Nevertheless,

the occurrence of univalents at metaphase I, which lag and divide equationally at anaphase I, apparently is the principal factor contributing to the production of aneuploid gametes in *D. glomerata*. Unequal distribution from quadrivalents seems to have been of infrequent occurrence. Only 2.5 per cent of the anaphase I sporocytes, in which laggards were not found, had other than a regular 14–14 distribution. At least part of the cases of unequal distribution probably could have resulted from random passage of metaphase I univalents to the poles.

It cannot be concluded that this behavior is typical for all or even for most autopolyploids. In fact, unequal distribution from multivalents has been reported commonly. RANDOLPH (19) found low frequencies of univalents at metaphase I and of laggards at anaphase I in autotetraploid maize, but unequal disjunction from quadrivalents occurred commonly. In autotetraploid *Lolium perenne* (unpublished data) both asynapsis and unequal disjunction occur in considerable frequency. COOPER'S (2) results with induced autopolyploid *Medicago sativa* also indicate that improper disjunction from quadrivalents may be an important source of laggards at anaphase I in some cases. It may be concluded that the feature of meiotic irregularity of greatest importance varies with the species, and that multivalent frequency alone will not always be a reliable criterion of stability and fertility of an autopolyploid.

In the present investigations both quadrivalent frequency and percentage of metaphase I sporocytes with univalents were significantly correlated with chiasma frequency; yet quadrivalent frequency was not correlated with frequency of metaphase I univalents. These seemingly anomalous results reveal an interesting relationship among these three characters. Judging from present knowledge of chromosome pairing, it is probable that numbers of quadrivalents are conditioned by two things, (*a*) number of chiasmata and (*b*) amount of chromosomal differentiation and other factors which tend to limit prophase synapsis to particular pairs, preventing multivalent association. The former should be positively and the latter negatively correlated with quadrivalent frequency. Univalents at metaphase I likewise should be affected by these two factors. Decreased multivalent association during prophase should result in more regular bivalent formation at metaphase I. The relative importance of this factor in conditioning quadrivalent frequency and asynapsis cannot be evaluated accurately with the available data. Nevertheless, the errors of estimate from the analysis of covariance indicated that there were differences among clones in quadrivalent frequency and also in metaphase I univalents which could not be attributed to differences in chiasma frequency. In instances where the factor of multivalent pairing in prophase was sufficiently important, it would condition a positive correlation between quadrivalent and metaphase I univalent frequencies. On the other hand, chiasma frequency was, as expected, positively correlated with number of quadrivalents and negatively correlated with incidence of unpaired chromosomes at metaphase I. This relationship would tend to cause a negative correlation between the latter two characters. In the material used in the present investigations, the two factors chiasma frequency and tendency for bivalent pairing in prophase were balanced in such a manner as to leave a correlation coeffi-

cient between quadrivalent and metaphase I univalent frequency which was not significantly different from zero.

The information regarding the interrelationships of frequencies of chiasmata, quadrivalents, and metaphase I univalents is useful as a guide in selecting for greater meiotic regularity in *Dactylis glomerata*. Selection for decreased quadrivalent frequency and increased chiasma frequency eventually should result in the greatest possible degree of regularity in meiosis. Selection for low quadrivalent frequency caused by low chiasma frequency would result in types with considerable asynapsis at metaphase I, and consequently with a high frequency of aneuploid gametes.

Previous studies (16, 17) found more laggards at anaphase I than could be accounted for by frequency of unpaired chromosomes at metaphase I. Similar results were obtained in the present investigations with five of the clones, while there were few or no differences in the other four. As suggested by MYERS and HILL (17), the greater incidence of laggards at anaphase I may have resulted either from their origin from some source other than metaphase I univalents or from greater difficulty in seeing all metaphase I univalents. The analysis of covariance indicated that all significant differences in anaphase I laggards were accounted for by variations in incidence of metaphase I univalents in these investigations. Therefore, the latter explanation seems more plausible.

Summary

1. Data on chromosomal association and behavior during meiosis were collected from three replications of each of six clones of *Dactylis glomerata* L. for two years, 1940 and 1941, and of three additional clones for one year, 1941. Statistically significant differences among clones were found for number of half-chiasmata per chromosome, average number of quadrivalents per sporocyte, frequency of unpaired chromosomes at metaphase I, frequency of lagging and dividing univalents at anaphase I, and frequency of micronuclei in the quartets. All characters were recorded in both years except chiasma frequency, which was recorded only in 1941. For all the characters recorded in both years the interaction of clones × years was significant, while the difference between years was significant only for frequency of univalents at metaphase I and for frequency of micronuclei in the quartets.

2. From analyses of covariance, correlation coefficients were calculated for the various characters. The correlation coefficients (r) of quadrivalent frequency with frequency of metaphase I univalents and with frequency of anaphase I laggards were not significant, while r of chiasma frequency with metaphase I univalents was negative and significant. Significant positive values of r were obtained between chiasma frequency and quadrivalent frequency, metaphase I univalents and anaphase I laggards, metaphase I univalents and micronuclei in the quartets, and anaphase I laggards and micronuclei in the quartets.

3. By means of analyses of covariance, it was found in all but one case that there were significant differences among plants in the dependent variable which could not be ascribed to its regression on the independent variable. In the exceptional case all significant differences among plants in occurrence of laggards at anaphase I could be accounted for by variations in frequency of metaphase I univalents.

4. It is suggested that in *D. glomerata* unequal distribution of chromosomes from quadrivalents is of minor importance in conditioning other meiotic irregularities. On the other hand, the presence of unpaired chromosomes at metaphase I which lag and divide longitudinally at anaphase I, tending to be left in the cytoplasm at telophase I or telophase II, appears to play a major role in that regard. Three of the nine clones had low frequencies of metaphase I univalents and consequently few laggards at anaphase I and few micronuclei in the quartets.

5. Significant differences among clones suggest the possibility of selecting lines of *D. glomerata* which would be more regular in meiosis and possibly more fertile. The relationships found among the various characters of meiosis serve as a guide for selection.

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LITERATURE CITED

1. COCHRAN, W. G., Long term agricultural experiments. Suppl. Jour. Roy Stat. Soc. 6:104–148. 1939.
2. COOPER, D. C., Artificial induction of polyploidy in alfalfa. Amer. Jour. Bot. 26:65–67. 1937.
3. DARLINGTON, C. D., Chromosome studies in *Fritillaria*. III. Chiasma formation and chromosome pairing in *F. imperialis*. Cytologia 2: 37–55. 1930.
4. ———, Recent advances in cytology. Blakiston, Philadelphia. Ed. 2. 1937.
5. FISHER, R. A., Statistical methods for research workers. Oliver and Boyd, London. Ed. 6. 1936.
6. GILES, NORMAN, Chromosome behavior at meiosis in triploid *Tradescantia* hybrids. Bull. Torr. Bot. Club 68:207–221. 1941.
7. IMMER, F. R., A study of sampling technic with sugar beets. Jour. Agr. Res. 44:633–647. 1932.
8. KAKHIDZE, N. T., Meiosis in inbred rye. Compt. Rend. (Doklady) Acad. Sci. U.R.S.S. 25:68–70. 1939.
9. KOSTOFF, D., Evolutionary significance of chromosome length and chromosome number in plants. Biodynamica 51:1–14. 1939.
10. MÜNTZING, ARNE, Quadrivalent formation and aneuploidy in *Dactylis glomerata*. Bot. Not. 1933:198–205. 1933.
11. ———, The evolutionary significance of autopolyploidy. Hereditas 21:263–378. 1936.
12. ———, The effects of chromosomal variation in *Dactylis*. Hereditas 23:113–235. 1937.
13. MYERS, W. M., Variations in chromosomal behavior during meiosis among plants of *Lolium perenne* L. Cytologia 11:388–406. 1941.
14. ———, Analysis of non-heritable variations in seed set under bag among plants of *Dactylis glomerata* L. Jour. Amer. Soc. Agron. 34:1114–1124. 1942.
15. ———, Cytological and genetical analysis of chromosomal association and behavior during meiosis in hexaploid timothy (*Phleum pratense* L.). Jour. Agr. Res. In press.
16. MYERS, W. M., and HILL, HELEN D., Studies of chromosomal association and behavior and the occurrence of aneuploidy in autotetraploid grass species, orchard grass, tall oat grass, and crested wheatgrass. BOT. GAZ. 102:236–255. 1940.
17. ———, and ———, Variations in chromosomal association and behavior during meiosis among plants from open-pollinated populations of *Dactylis glomerata*. BOT. GAZ. 104:171–177. 1942.
18. MYERS, W. M., and POWERS, LEROY, Meiotic instability as an inherited character in varieties of *Triticum aestivum*. Jour. Agr. Res. 56:441–452. 1938.
19. RANDOLPH, L. F., Cytogenetics of tetraploid maize. Jour. Agr. Res. 50:591–605. 1935.
20. ———, An evaluation of induced polyploidy as a method of breeding crop plants. Amer. Nat. 75:347–363. 1941.
21. SAX, KARL, Variation in chiasma frequencies in *Secale*, *Vicia*, and *Tradescantia*. Cytologia 6: 289–293. 1935.
22. SNEDECOR, G. W., Statistical methods. Collegiate Press, Ames, Iowa. 1938.
23. UPCOTT, MARGARET, The cytology of triploid and tetraploid *Lycopersicum esculentum*. Jour. Gen. 31:1–19. 1935.

CYTOLOGICAL STUDIES IN RELATION TO THE CLASSIFICATION OF THE GENUS CALOCHORTUS. III.

J. M. BEAL AND MARION OWNBEY

In two previous papers (1, 2) the number and morphology of the chromosomes of twenty-eight species and three varieties of the genus *Calochortus* (Liliaceae), as recognized in OWNBEY'S monograph (5), have been reported. In a subsequent study of megasporogenesis and megagametophyte development in fourteen species of this genus, CAVE (3) has reported chromosome numbers for these species as determined by BURRELL (previously unpublished). The numbers of three of these species, *C. pulchellus* n = 10, *C. umbellatus* n = 10, and *C. palmeri* n = 7, had not been reported before, and one species, *C. weedii* n = 9, had been investigated only in the variety *vestus*, which showed 2n = 18 in root tips. The numbers recorded in CAVE'S paper are in complete agreement with those previously reported for the species studied by BEAL (1, 2) and by NEWTON (4), except for the entity listed as *C. davidsonianus*, for which a chromosome number of n = 14 is reported. *C. davidsonianus* was considered by OWNBEY (5) to be a minor geographical variant of *C. splendens*, a species which BEAL found to possess 2n = 14 chromosomes. Both counts have been confirmed by OWNBEY (unpublished) in material of known geographical origin. Thus it appears that the cytological difference between *C. davidsonianus* and *C. splendens* is more striking than the morphological difference. Whether or not *C. davidsonianus* should be considered a full species or merely a tetraploid variety of *C. splendens* need not be decided at the present time. It is sufficient to point out that the two are morphologically, cytologically, and geographically distinguishable.

The present paper deals with three additional species and three varieties not heretofore studied, together with *C. palmeri* on which CAVE reported but for which she figured no chromosomes.

The bulbs used in these studies were secured by OWNBEY, either through his own field work or from correspondents. The geographical source of each bulb collection and the collector's name in each instance are given in a later section of this paper. In so far as has been practicable, a herbarium specimen from each collection has been preserved. Mitotic studies were carried out by BEAL according to the method described earlier (1, 2). Meiosis in pollen mother cells was investigated by OWNBEY by means of the aceto-carmine smear technique, using anthers from plants grown out of doors at Pullman, Washington.

In conformity with earlier papers, the sequence of sections, subsections, and species followed is that of OWNBEY'S monograph (5).

SECTION I. EUCALOCHORTUS

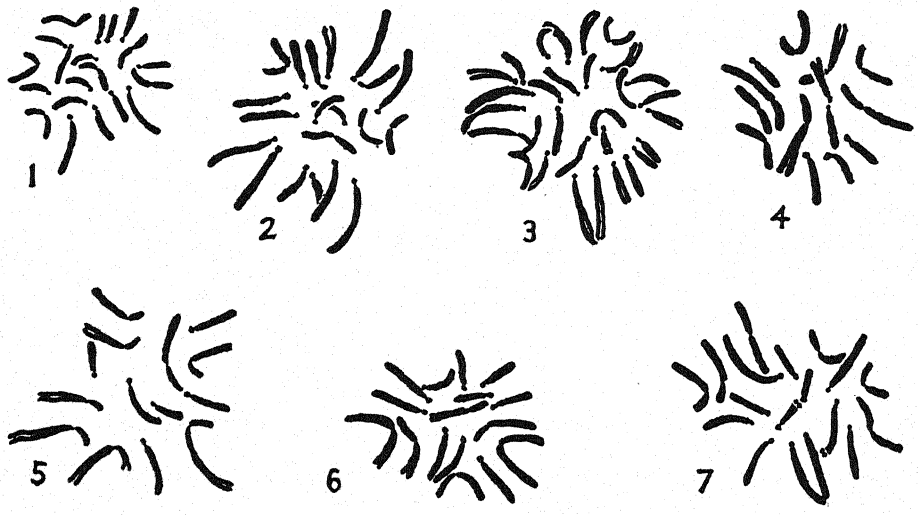
Subsection 2. Eleganti

C. elegans var. *nanus* Wood (*C. elegans* var. *oreophilus* Ownbey).—Diploid, 20 chromosomes (fig. 1); 10 bivalents at metaphase I of meiosis (not figured). The karyotype of this entity resembles more closely those of *C. lobbii* and *C. apiculatus* than it does those of *C. elegans* and *C. tolmiei*, thus suggesting that the variety *nanus* may not be so closely allied to *C. elegans* as its very similar morphology might seem to indicate. Further evidence on the genetic distinctness of these two entities has been derived from reciprocal cross pollinations between them (OWNBEY, unpublished). No hybrid seeds have been secured, although

both entities produce abundant seed under garden conditions if open-pollinated.

The material of this variety (*Ownbey & Brown 2429*) was collected in open coniferous forest along the ridge south of Gunsight Peak in the mountains west of Yreka, Siskiyou County, California. WOOD's original specimens on which this variety was based were secured in the "mountains w. of Yreka," doubtless from the same station or near it. Thus the identity of the single specimen of the original collection now preserved at the Gray Herbarium seems clearly established. It has been confused (OWNBEY, 5; and earlier investigators) with a more southern geographical variant of *C. coeruleus* (or possibly of *C. tolmiei*). The collection of OWNBEY and BROWN clearly identifies the type collection of *C. elegans* var. *nanus* with the entity recently described by OWNBEY (5) as *C. elegans* var. *oreophilus*, and the former name must therefore replace the latter as the accepted one for the entity.

Additional chromosome counts, all n = 10, have been made by OWNBEY on the following collections of this variety, all from Siskiyou County, California: *Ownbey & Meyer 2172*, from the west slope of the Siskiyou Mountains, northwest of Hornbrook; *Ownbey & Meyer 2210* and *2211*, both from the mountains southwest of Yreka; and *Ownbey & Ownbey 1748*, from the summit of the Siskiyou Mountains, $2\frac{1}{2}$ miles northwest of Dry Lake Lookout. The last is the type collection of *C. elegans* var. *oreophilus*.



FIGS. 1–7.—Root-tip metaphases: fig. 1, *C. elegans* var. *nanus*, 2n = 20 (*Ownbey & Brown 2429*); fig. 2, *C. longebarbatus*, 2n = 20 (*Ownbey & Ownbey 2345*); fig. 3, *C. longebarbatus* var. *peckii*, 2n = 30 (*Ownbey & Ownbey 2360*); fig. 4, *C. flexuosus*, 2n = 14 (*Clokey 5872*); fig. 5, *C. palmeri*, 2n = 14 (*Ownbey & Ownbey 1675*); fig. 6, *C. kennedyi* var. *munzii*, 2n = 16 (*Cutler 4764*); fig. 7, *C. ambiguus*, 2n = 18 (*Leyendecker*). ×2450.

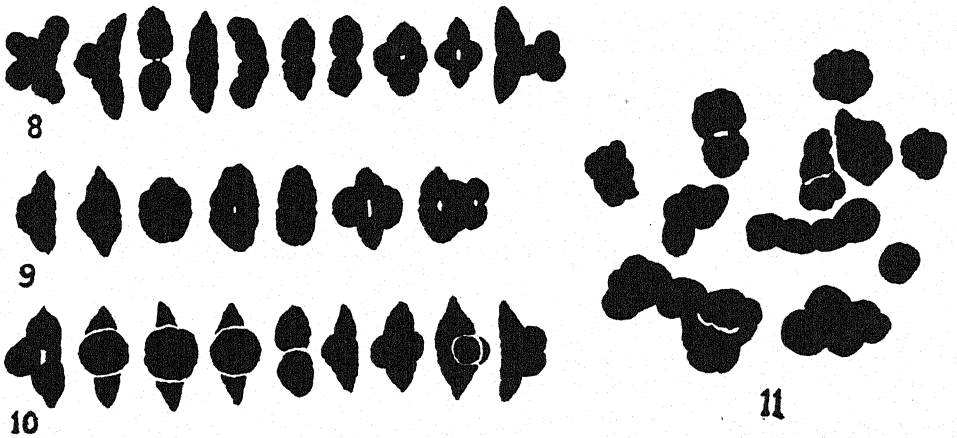
Subsection 4. Nitidi

C. longebarbatus Watson.—Diploid, 20 chromosomes (fig. 2); 10 bivalents at metaphase I of meiosis (fig. 8). The chromosomes of this species conform closely in their morphology to those of other species of the subsection Nitidi, especially to those of *C. persistens* Ownbey. Bulbs

of this latter species were secured from Mr. CARL PURDY under the name "*C. greenei*," and they were reported under that name in a former study (1) as possessing 2n = 20 chromosomes.

The present geographical distribution of *C. longebarbatus* is highly disrupted. On the basis of gross discontinuities alone, the distributional area now occupied by this species may be broken up into four or five well-separated districts. The first and largest of these covers southwestern Yakima and western Klickitat counties in Washington, and adjacent Hood River County, Oregon. This is the only area in which the species is more than occasionally met with. It was from this area also that the original material on which this species was based was collected. The record of its occurrence in Whitman County, Washington, 150 miles to the northeast, across the inhospitable semideserts of central Washington, is suspected to be in error. Intensive search at the station from which it has been reported has, so far at least, failed to disclose its occurrence there at the present time.

A second locality in which *C. longebarbatus* occurs (but here as the var. *peckii* to be described below) is in the Ochoco National Forest east of Prineville in Wheeler and Crook counties, Oregon, and a third in the same state is in the mountains about the headwaters of the Sycan River in Klamath or Lake County. Farther south it reappears in a small area in southwestern Modoc and northeastern Shasta counties, California. All these districts are separated by many miles of inhospitable habitats in which the species does not occur at the present time.



FIGS. 8–11.—Meiotic metaphase I in pollen mother cells: figs. 8–10, individual bivalents from single cell drawn separately in side view; fig. 11, polar view of metaphase plate. Fig. 8, *C. longebarbatus*, n = 10 (*Ownbey & Ownbey 2345*); fig. 9, *C. palmeri*, n = 7 (*Ownbey & Ownbey 1675*); fig. 10, *C. ambiguus*, n = 9 (*Leyendecker*); fig. 11, *C. longebarbatus* var. *peckii*, pairing irregular (*Ownbey & Ownbey 2360*). ×2700.

The material of diploid *C. longebarbatus* came from almost the opposite extremes of its known geographical range. *Ownbey & Ownbey 2345* was collected about 13 miles northwest of Goldendale, Klickitat County, Washington, a few miles east of the type locality (in "Falcon Valley") and across the canyon of the Klickitat River from it. A second collection (*Constance & Beetle 2761*) from along Satus Creek, 21 miles south of Toppenish, Yakima County, investigated by OWNBEY, likewise shows 10 bivalents at

metaphase I of meiosis. From the opposite end of the range, *Ownbey & Brown 2417* from Goose Valley, near Burney, Shasta County, California, has been investigated only by BEAL. The bulbs of this lot were collected too early in the season and were so weakened that they did not produce a sufficient number of buds for meiotic studies during their first year under cultivation. No significant differences have been noted between the karyotype of this collection and that of *Ownbey 2345*. This is rather remarkable in view of the possibility that the populations at the two stations doubtless have been separated for a very long time—perhaps for a million years.

C. longebarbatus var. *peckii* Ownbey.[1]

[1] **Calochortus longebarbatus** var. **peckii** Ownbey, var. nov., folio basilari incurvo relative longiore et angustiore basilariter rubro-purpureo; caule humiliore; floribus crateriformibus, petalis latioribus brevioribus prope glandulam abrupte flectis, cuneolo librato et limbo erecto; aliter similis speciei.—OREGON: grown from bulbs of *Ownbey & Ownbey 1800* from grassy margin of wet meadow, headwaters of Marks Creek, Ochoco National Forest, Wheeler County (TYPE, in the Herbarium of the State College of Washington); also from bulbs of *Ownbey & Ownbey 2360* from grassy places under pines, along North Fork of Crooked River, just above the mouth of Deep Creek, S. 27, T. 14 S., R. 22 E., Crook County. Named in honor of its discoverer, Professor MORTON E. PECK of Willamette University, who had collected it at the Marks Creek station (*Peck 17200*), and who directed the writer (OWNBEY) to the exact locality during the summer of 1938.

One familiar with herbarium material only might easily minimize the morphological characters which distinguish the variety *peckii* from typical *C. longebarbatus*, but when living material is examined, it may be recognized at nearly all stages of development. Before flowering, the relatively longer and narrower basal leaf, which is reddish at the base and arcuate inward, is characteristic. Later it may be recognized by its lower stature and especially by its bowl-shaped flowers. The latter are particularly striking. The petals, which are relatively broader and shorter than in the species, are sharply bent at the gland, with the claw horizontal and the limb vertical. This characteristic flower shape is found elsewhere in the genus only in the related but amply distinct *C. greenei*. Flowers of typical *C. longebarbatus* are obconic campanulate.

—Triploid, 30 chromosomes (fig. 3). The occurrence of each of the chromosome forms in triplicate suggests that this variety is an autotriploid. At metaphase I of meiosis, however, the association of the chromosomes into configurations other than trivalents reveals that this is not entirely the case. In most of the cells examined the chromosomes were formed into eleven or twelve groups (fig. 11), some of which were bivalents, some trivalents, and some quadrivalents. In other cells configurations containing apparently more than four chromosomes, and also an occasional univalent, may occur. In none of the meiotic material examined has it been possible to account for all the 30 somatic chromosomes with certainty.

Notwithstanding the great irregularities at meiosis, development proceeds normally until after the tetrad has broken apart to form the individual microspores. Thereafter maturation is arrested at various stages, until at anthesis between 80 and 90 per cent of the pollen is visibly imperfect. That only a small amount of the pollen which matures is actually functional was indicated when the anther contents were applied to covered stigmas of typical *C. longebarbatus*. This cytologically regular material (n = 10) from Klickitat County, Washington, produces an abundance of seed under usual conditions, but only a few abortive seeds, mostly without embryos, resulted when twelve flowers were pollinated with an excess of pollen from var. *peckii*. Furthermore, in the fifty-one fruits which have been allowed to develop on var. *peckii* during the last two seasons, not a single seed was formed, although an abundance of viable pollen must have reached the stigmas from the Klickitat County collection growing by its side. Similar observations were made

in the field in 1940, when not a single seed was found although many fruits were examined.

The probability that *C. longebarbatus* var. *peckii* is unable to reproduce itself by seed seems well enough established. To most species of the section Eucalochortus this would be a fatal handicap, for, although all of them—so far as is known—reproduce vegetatively through division of the bulb, this is far too slow a process in most cases to maintain the species. *C. longebarbatus*, however, is one of the two species of this section which regularly produce bulblets in the leaf axils at or below the surface of the ground. With this effective means of vegetative reproduction, *C. longebarbatus* var. *peckii* has not only maintained itself since its origin but has apparently been able to increase its numbers. If all individuals of this variety represent only one clone, as is here suggested, then its distribution is indeed of significance. At the present time it is known only from the two localities cited. These are about 18 miles apart and are now separated by an expanse of habitats in which the variety does not grow. Assuming that the original point of dispersal was halfway between the two known stations, it has been necessary for this variety to migrate at least 9 miles since its origin. If it were to move at an average rate of 1 inch per year in a straight line (an exceedingly generous estimate), it would take well over half a million years to migrate 9 miles. Perhaps in a case of this kind transport by rodents, or possibly by water (although the two stations are on different drainage systems), would be more important than mere growth. The probability remains, however, that *C. longebarbatus* var. *peckii* is very old. Considering this, the cytological and morphological uniformity of the plants from the two localities becomes truly remarkable. Is it possible that evolution has been at a standstill in this clone for 500,000 years? This should be expected, of course, since the genes are presumably in triplicate, and there is no provision for their reassortment through reduction division. Barring dominant gene mutations, the rate of phenotypic change should approximate the cube of the mutation rate of a given gene. This would impose a handicap of great evolutionary consequence on those characters governed by even the most labile of the genes. If the variety *peckii* is old, then meiotically-regular *C. longebarbatus* must be older. Although var. *peckii* appears to have remained constant since its origin, it seems unlikely that sexually-reproducing *C. longebarbatus* would have done likewise during the same period of time. It seems possible, therefore, that here in var. *peckii* are some of the genes which have been eliminated from *C. longebarbatus* in the last 500,000 years. Hybrids, if they can be obtained, and apparently they can, should be very interesting.

The resemblances between *C. longebarbatus* var. *peckii* and *C. greenei* may also be of significance in the interpretation of the phylogeny of the Eucalochorti. As OWNBEY (5) has pointed out, the center of dispersal of this section appears to be coincident with the ancient Klamath land area of northern California and southwestern Oregon. This does not mean that the present-day species originated on and migrated out from the Klamath area, but that at some time the ancestor or ancestors of these species occupied this area as a refuge during an unfavorable era. *C. greenei* now occurs only within that area, but *C. longebarbatus* has a much disrupted distribution, extending from Shasta County, California, to Washington. Geographically, *C. longe-*

barbatus var. *peckii* occurs between *C. greenei* and typical *C. longebarbatus* in southern Washington and northern Oregon. Its resemblance to *C. greenei* suggests that it may have retained certain genes in common with that species which have been lost by its sexually-reproducing relative. The few collections of *C. longebarbatus* from southern Oregon and northern California are all old and are inadequate for determining whether or not they are identical with the material from farther north. Living material secured in Goose Valley, Shasta County, during the summer of 1941 (*Ownbey & Brown 2417*) should be of assistance in this connection.

Section II. Mariposa

Subsection 5. Venusti

C. flexuosus Watson.—Diploid, 14 chromosomes (fig. 4). Karyotype similar to that of *C. catalinae*, with both species showing only 4 chromosomes with subterminal centromeres, and the remaining 10 have median or submedian centromeres. The two species are evidently closely related, both cytologically and morphologically.

The material of this species was collected by Mr. Ira W. Clokey (no. 5872) on gravelly slopes and hillsides at Glendale Junction, about 50 miles northeast of Las Vegas, Clark County, Nevada.

C. palmeri Watson.—Diploid, 14 chromosomes (fig. 5); 7 bivalents at metaphase I of meiosis (fig. 9). The chromosomes of this species conform closely in morphology to those of *C. splendens*, with which it is closely allied otherwise. Both show 6 chromosomes with subterminal centromeres, and the remaining 8 have similar forms and centromere positions in the two species.

The material of *C. palmeri* (*Ownbey & Ownbey 1675*) was collected in a grassy swale along the south side of Big Bear Lake, San Bernardino Mountains, San Bernardino County, California.

Subsection 7. Nuttalliani

C. kennedyi var. *munzii* Jepson.—Diploid, 16 chromosomes (fig. 6). The karyotype of this variety is scarcely distinguishable from that of the species, of which it is perhaps no more than a color form.

Bulbs of this variety were removed from herbarium specimens collected by Dr. Hugh C. Cutler (no. 4764) on a grassy plain, 8 miles southeast of Sonoita, Santa Cruz County, Arizona. No material was available for meiotic studies.

C. ambiguus (Jones) Ownbey.—Diploid, 18 chromosomes (fig. 7); 9 bivalents at metaphase I of meiosis (fig. 10). The karyotype of this species is almost identical with that of *C. gunnisoni*, and the two species are otherwise very similar. On the basis of their very close morphological resemblance, these two species, together with a variety of *C. gunnisoni*, were placed together by Ownbey (5) to constitute the subsection Gunnisoniani. The cytological evidence now supports that derived from morphology in indicating the validity of this subsection.

The material of *C. ambiguus* was collected about 1 mile west of Cloverdale, in the southwestern corner of Hidalgo County, New Mexico, by Mr. P. J. Leyendecker. Additional material from 2 miles southeast of Sonoita, Santa Cruz County, Arizona, collected by Dr. Hugh C. Cutler, was investigated only by Beal. There appears to be little or no karyotypic difference between the two collections.

Discussion

Including the species and varieties investigated in the present study, a total of thirty-six species and six varieties of

the genus *Calochortus* have been examined cytologically. This total includes *C. davidsonianus*, *C. kennedyi* var. *munzii*, and *C. longebarbatus* var. *peckii*, which were not included among the fifty-seven species and twelve varieties of this genus accepted by OWNBEY (5). In table 1 the chromosome numbers of the species and varieties which have been investigated cytologically are summarized. In order to demonstrate the close correlation between chromosome number and systematic position existing in this genus, the sequence of sections, subsections, species, and varieties is that of OWNBEY's monograph. In each case the name of the investigator or investigators responsible for the count is indicated in the right-hand column.

Of the twenty-one species and five varieties now recognized in the section Eucalochortus, eighteen species and three varieties have been investigated cytologically. All of these have shown 10 as the basic chromosome number. All are diploid except *C. pavonaceus* and *C. uniflorus*, which are tetraploid, and *C. longebarbatus* var. *peckii*, which is triploid.

Among the twenty-two species (including *C. davidsonianus*) and seven varieties (including *C. kennedyi* var. *munzii*) of the section Mariposa, sixteen species and two varieties have been cytologically examined. All ten species of the subsection Venusti which have been investigated show 7 as the basic chromosome number, at least in part. Eight of these species are diploid and two are tetraploid. In two species a basic chromosome number other than 7 is found in some localities. In *C. superbus*, two cytological races, one n = 6 and one n = 7, are scarcely to be distinguished on tangible morphological characters. The same is true of *C. luteus*, where there is only a cytological distinction between the race with n = 7 and that with n = 10 chromosomes. In both of these cases major cytological changes have taken place, apparently so recently that there has been no time for genetic divergence. In other words, here are two cases in which genetic isolation through gross cytological change has preceded morphological differentiation. It may be assumed that, if the two races in each instance persist long enough, such morphological differentiation may appear. Indeed, such may have been the origin of the natural groups of *Calochortus* now characterized by a distinctive chromosome number.

Like most of the species of the subsection Venusti, the single species included in the subsection Macrocarpi has a basic chromosome number of 7. In the subsection Nuttalliani, however, the three species and two varieties for which a chromosome number has been reported have a basic number of 8. *C. nuttallii* var. *aureus*, the only tetraploid of the lot, is as distinct morphologically, cytologically, and geographically as is *C. davidsonianus*. It may be well to point out here that the basic number of 8 is not constant for the entire subsection Nuttalliani (OWNBEY, unpublished).

The two species included in the subsection Gunnisoniani, section Mariposa, have a basic chromosome number of 9, with no known polyploidy. It is probably significant that these two species are more similar morphologically to the species of the subsection Nuttalliani (n = 8) than they are to species of the subsections Venusti or Macrocarpi (n = 7). They are, of course, very different from the species of the section Cyclobothra (n = 9) and of the section Eucalochortus (n = 10). If 7 is the ancestral number for the genus, as has been suggested by BEAL (1), this would indicate that these higher basic numbers have been derived

TABLE 1

REPORTED CHROMOSOME NUMBERS IN THE GENUS CALOCHORTUS, WITH SPECIES AND VARIETIES ARRANGED INTO SUBSECTIONS AND SECTIONS ACCORDING TO THEIR NATURAL AFFINITIES AS INDICATED BY MORPHOLOGICAL AND CYTOLOGICAL EVIDENCE

Section	n	2n	Investigator
Section I: Eucalochortus			
Subsection 1. Pulchelli			
C. amoenus	10*	20	Beal, Burrell
C. albus	10*	20	Beal, Burrell, Newton
C. pulchellus	10*	.....	Burrell
C. amabilis	10*	20	Beal, Newton
Subsection 2. Eleganti			
C. monophyllus (C. benthami)	10*	.....	Burrell, Newton
C. tolmiei	10*	20	Beal
C. elegans	(10)*	20	Beal
C. elegans var. selwayensis	(10)*	20	Beal
C. elegans var. nanus†	10	20	Beal and Ownbey
C. lobbii	(10)*	20	Beal
C. apiculatus	10*	20	Beal
Subsection 3. Nudi			
C. umbellatus	10*	.....	Burrell
C. uniflorus	20*	40	Beal
C. nudus	(10)*	20	Beal
Subsection 4. Nitidi			
C. longebarbatus	10	20	Beal and Ownbey
C. longebarbatus var. peckii	?	30	Beal and Ownbey
C. pavonaceus‡	20*	40	Beal
C. nitidus	(10)*	20	Beal
C. howellii	10*	20	Beal
C. lyallii	10*	20	Beal
C. persistens	(10)*	20	Beal (as C. greenei)
Section II: Mariposa			
Subsection 5. Venusti			
C. catalinae	7*	14	Beal, Newton
C. flexuosus	.....	14	Beal and Ownbey
C. palmeri	7	14	Beal and Ownbey, Burrell
C. splendens	7*	14	Beal
C. davidsonianus	14*	.....	Burrell
C. venustus	7*	14	Beal, Burrell, Newton
C. superbus	6,* 7*§	12, 14§	Beal
C. vestae	14*	28	Beal, Burrell, Newton
C. luteus	7,* 10*	14, 20, 21	Beal, Burrell, Newton
C. leichtlinii	(7)*	14	Beal
Subsection 6. Macrocarpi			
C. macrocarpus	(7)*	14	Beal
Subsection 7. Nuttalliani			
C. nuttallii	(8)*	16	Beal
C. nuttallii var. aureus	.....	32	Beal
C. kennedyi	.....	16	Beal
C. kennedyi var. munzii	.....	16	Beal and Ownbey
C. clavatus	8*	16	Beal, Burrell

* Count independently confirmed by OWNBEY (unpublished). Numbers to be inferred from the mitotic count, but previously unreported, are inclosed in parentheses.

† *C. elegans* var. *oreophilus* Ownbey, not *C. coeruleus* var. *nanus* Ownbey.

‡ Use of the name *C. douglasianus* for this entity cannot be maintained.

§ Reported as *C. luteus* var. *oculatus*, a misdetermination.

TABLE 1—*Continued*

Section	n	2n	Investigator
Subsection 8. Gunnisoniani			
C. ambiguus	9	18	Beal and Ownbey
C. gunnisoni	(9)*	18	Beal
Section III: Cyclobothra			
Subsection 9. Weediani			
C. plummerae	9*	18	Beal, Burrell, Newton
C. weedii	9*	.....	Burrell
C. weedii var. vestus	(9)*	18	Beal
Subsection 11. Barbati			
C. barbatus	(18)*	36	Beal

independently upon at least three different occasions. This hypothesis is adequately supported by geographical evidence. The section Eucalochortus is primarily northwestern in distribution, with a center in northern California. The section Cyclobothra is found only in southern California and Mexico. The two species of the subsection Gunnisoniani are limited in their distribution to the Rocky Mountains and the deserts of the southwest. In only one instance do species belonging to any two of these entities occupy the same area. In southern California, the range of *C. albus* (section Eucalochortus) includes territory occupied by species belonging to the subsection Weediani (section Cyclobothra).

All the species of the section Cyclobothra which have been examined cytologically show 9 as the basic chromosome number. There is one known case of tetraploidy. Most of the species of this group are Mexican, however, and for this reason living material has been unavailable for cytological study. It is hoped that anyone having opportunity to collect bulbs of one or more of these species will get in touch with the writers.

The data presented here show conclusively that a natural taxonomic system of classification is possible in the genus *Calochortus* which satisfies the requirements of the cytological, geographical, and morphological evidence. This system closely parallels that outlined by OWNBEY (5), wherein the species are arranged according to the maximum correlation of morphological characters into twelve subsections and three sections. Resemblance in chromosome number and karyotype morphology between species so associated is marked. Each species group, whether sectional or subsectional, is characterized by a geographical distribution differing from that of any other species group. Thus it seems clearly established that OWNBEY'S arrangement of the genus *Calochortus* into sections and subsections accurately indicates actual phylogenetic relationships.

Summary

1. Chromosome counts for the following species and varieties of *Calochortus* are reported: *C. elegans* var. *nanus*.—Diploid, 20 chromosomes; 10 bivalents at metaphase I of meiosis. *C. longebarbatus*.—Diploid, 20 chromosomes; 10 bivalents at metaphase I of meiosis. *C. longebarbatus* var. *peckii*.—Triploid, 30 chromosomes; irregular pairing at metaphase I of meiosis. *C. flexuosus*.—Diploid, 14 chromosomes. *C. palmeri*.—Diploid, 14 chromosomes; 7 bivalents at metaphase I of meiosis. *C. kennedyi* var. *munzii*.—

Diploid, 16 chromosomes. *C. ambiguus.*—Diploid, 18 chromosomes; 9 bivalents at metaphase I of meiosis. All previously reported meiotic counts have been confirmed in material of known geographical origin and certain identity. Meiotic counts have been made in the following species and varieties for which this number might be inferred from the previously-reported mitotic count: *C. elegans*, 10 bivalents; *C. elegans* var. *selwayensis*, 10 bivalents; *C. lobbii*, 10 bivalents; *C. nudus*, 10 bivalents; *C. nitidus*, 10 bivalents; *C. persistens*, 10 bivalents; *C. leichtlinii*, 7 bivalents; *C. macrocarpus*, 7 bivalents; *C. nuttallii*, 8 bivalents; *C. gunnisoni*, 9 bivalents; *C. weedii* var. *vestus*, 9 bivalents; *C. barbatus*, 18 bivalents (some irregularity).

2. *C. longebarbatus* var. *peckii*, a naturally occurring, sterile, triploid clone, is described, and its biology, relationships, and probable history are discussed in detail. The application of the name *C. elegans* var. *nanus* is established.

3. All species and varieties of the genus for which a chromosome number has been reported are arranged into subsections and sections according to the sequence of OWNBEY. The section Eucalochortus is characterized by a chromosome base number of 10, with two tetraploid species and a triploid variety. The subsection Venusti of the section Mariposa has 7 as a base number, with secondary base numbers of 6 and 10 in two species in part of their range. There are two tetraploid species in this subsection. The subsection Macrocarpi of this section likewise has 7 as a base number, with no known exceptions. All species of the subsection Nuttalliani for which a chromosome number has been reported have 8 as a base number. There is one tetraploid variety. Finally, in the subsection Gunnisoniani of the section Mariposa, 9 is the only reported base number. In the section Cyclobothra the few species and the varieties which have been investigated cytologically all show 9 as the base number, with a single known instance of tetraploidy.

4. It is concluded—on the basis of morphological, cytological, and geographical criteria—that the classification as outlined by OWNBEY accurately indicates actual phylogenetic relationships.

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LITERATURE CITED

1. BEAL, J. M., Cytological studies in relation to the classification of the genus *Calochortus*. BOT. GAZ. 100:528–547. 1939.
2. ———, Cytological studies in relation to the classification of the genus *Calochortus*. II. BOT. GAZ. 102:810–811. 1941.
3. CAVE, MARION S., Megasporogenesis and embryo sac development in *Calochortus*. Amer. Jour. Bot. 28:390–394. 1941.
4. NEWTON, W. C. F., Chromosome studies in *Tulipa* and some related genera. Jour. Linn. Soc. Bot. London 47:339–354. 1926.
5. OWNBEY, MARION, A monograph of the genus *Calochortus*. Ann. Missouri Bot. Gard. 27:371–560. 1940.

MEIOSIS IN AUTOTETRAPLOID SECALE CEREALE[1]

JOSEPH G. O'MARA[2]

Introduction

Cytological studies in induced autotetraploids seem to be important for an understanding of the apparent stability and uniformity of the autotetraploid condition and of both the natural and practical consequences of its induction. While the theoretical possibilities of autotetraploidy have been determined adequately, the actual results seem to deserve more attention than they have had. The present investigation has been directed toward determining the meiotic anomalies which occur in an induced autotetraploid, *Secale cereale*.

Most of the cytological investigations on autotetraploids have been made in species which have been autotetraploid for an undetermined number of generations. Consequently, the simple unmodified autotetraploid meiosis cannot be accurately described, the meiotic process having undergone selection for many generations. Furthermore, nothing can be known in many autotetraploids of the origin of the tetraploid condition; under some circumstances the tetraploid should be "homozygous" and under others it may be "heterozygous." It is possible that polyploids which differ in this respect, or in other possible directions, would act differently at the reduction divisions. The simplest case for analysis would seem to be that of an autotetraploid which had arisen by somatic doubling within a generation or two of the time at which the observations were made.

MATERIAL AND METHODS.—The material used for these observations was autotetraploid *Secale cereale* with spring habit. It was derived directly from the diploid strain by somatic doubling, presumably during the seedling stage. The original diploid was examined many times—in connection with other work as well as with the present— and found to be in no way unusual at meiosis. All the preparations were made from material which had been fixed from 2 days to several weeks in a solution of 6 parts ethyl alcohol, 2 parts chloroform, and 1.5 parts acetic acid. This fixed material was kept in a refrigerator until used. Such treatment obscures the finer meiotic details which have been shown to be present in this material, especially the chromosome coils (3), but it has the advantage of affording large quantities of microsporocytes over a long period. The preparations were made with aceto-carmine in the usual manner. All photographs were taken with a Leitz Makam photomicrographic camera in conjunction with apochromatic objectives. Both the diploid and tetraploid rye were given the writer by Dr. KARL SAX, the latter when it was but one meiosis removed from its somatic doubling.

Experimentation

GENERAL OBSERVATIONS

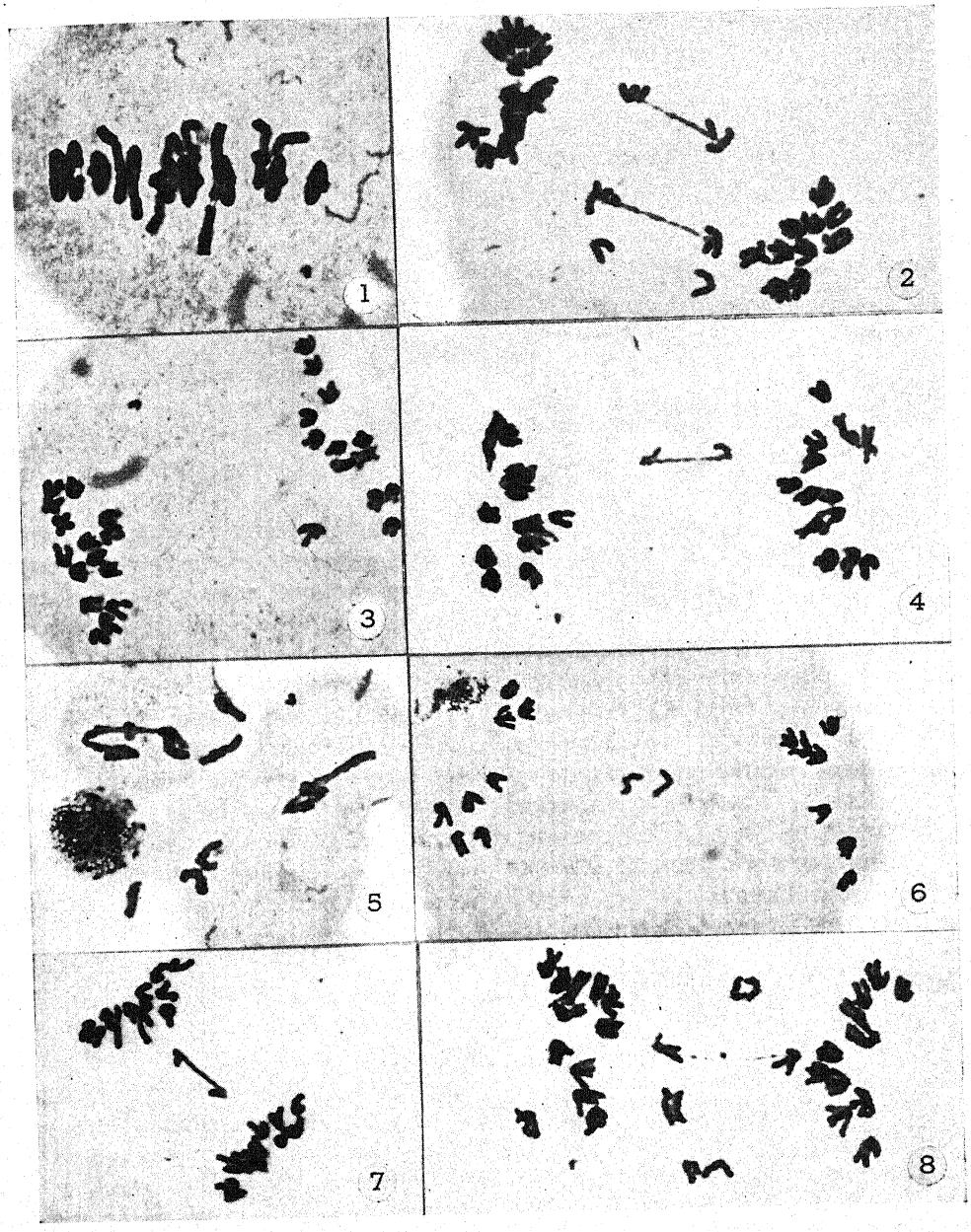
The autotetraploid microsporocyte of *S. cereale* is very similar to that of other described autotetraploids—*Zea mays*, *Tradescantia virginiana*, etc. At diakinesis and metaphase the chromosomes occur as univalents, bivalents, trivalents, and quadrivalents. A metaphase which contains various chromosome associa-

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tions may be seen in figure 1. Such associations (not all present in the figure) are ring and rod bivalents, chains of three, and chains and rings of four. While these are the usual configurations, more complex and unusual types infrequently occur, that is, ring trivalents, triple arc trivalents, and interlocked quadrivalents. The univalents seem to occur in random positions in the microsporocyte when the associated chromosomes are oriented on the metaphase plate. After the associated chromosomes have disjoined and passed toward the poles, the univalents pass to the plate and split, each half passing to its nearer pole from one to several chromosome lengths behind the main group of anaphase chromosomes. Many such univalents may be seen in figures 2, 6, and 8. These photomicrographs were taken of cells which had been flattened sufficiently to bring all the chromosomes into one focus; consequently the distances between the chromosomes are exaggerated along the division axis in side views. It is therefore probable that a degree of lagging is indicated for the univalents which greatly exceeds that found in the unaltered material. At this point, early telophase, various degrees of difference become manifest in the time consumed by the univalents in reaching the plate. Some univalents split early enough to be included in the telophase nuclei, while others are left in the cytoplasm, where they form micronuclei. Stages between these extremes are frequent, and many univalents are but partially included in the telophase nuclei, the section nearest the plate being excluded by the nuclear membrane which seems to pass through the chromosome arm. At division II the process is usually normal in appearance, except for the behavior of the previously univalent chromosomes, which, having effectively but one thread, cannot split as the other chromosomes and so pass to the poles at random. Owing to the delay with which these chromosomes are oriented on the plate, various degrees of lagging now occur, and as a consequence many micronuclei are formed. These micronuclei are most clear in the quartets, where they show themselves to be small spheres, often very different in diameter and consequently far more different in volume. The pollen has irregularities in size and shape consistent with the observable meiotic anomalies and characteristic of most other autotetraploids.

These are the usual events at meiosis in the tetraploid. One exception is to be found in nonlagging univalents—univalents which do not reach the metaphase plate and split. These are included wholly in the telophase nuclei, or (rarely) are so distant from the telophase mass that they form micronuclei at interphase. It could not be determined to what extent this failure to reach the plate occurred, since once the disjunction has occurred it is impossible to decide how a single isolated chromosome reached the polar end of a cell. This is so because some chromosomes appear to reach the pole before their partners, and because in flattening the preparations the relative positions of the chromosomes are very often, if not always, changed. However, from many extreme instances in which the main body of chromosomes was in the anaphase stage and the univalents were behaving as characteristic laggards (splitting on the plate), some univalents were observed at or near the poles with their arms pointed away from the metaphase plate, a position which would seem to indicate that the chromosome had not been involved in any way in the division. It seems probable, therefore, that the anaphase laggards do not represent all the



FIGS. 1–8.—Fig. 1, metaphase plate showing characteristic chromosome associations; fig. 2, anaphase I with inversion or duplication bridges; fig. 3, anaphase I with a 15–13 distribution; fig. 4, univalent bridge at anaphase I; fig. 5, two contiguous cells, each deficient for three complete chromosomes; contiguity suggests that complete mitosis was achieved in spite of this large deficiency; fig. 6, anaphase I showing large loss of chromosomes and split laggard; fig. 7, bridge at anaphase II; fig. 8, anaphase I with three laggards and inversion or duplication bridge with fragment.

metaphase univalents but only those so placed as to be drawn into the spindle. A measure of the differences in frequency between metaphase univalents and anaphase laggards could be secured by tabulating the numbers which occur at each stage. This would be very simple for the anaphase laggards, which occur with precise structure and position; but the metaphase univalents may be concealed in the chromosome mass on the metaphase plate, behind that mass, or above it. For obvious optical reasons it is not always, perhaps not often, safe to conclude whether a suspected chromosome is loosely associated with a partner, intimately involved in a multivalent, or merely mechanically entwined, in well-flattened preparations. The validity of this decision is confirmed by the data secured in an attempt to determine this difference in frequency between anaphase laggards and metaphase univalents by such a tabulation. In many plants more anaphase laggards were counted than could be accounted for by observable metaphase univalents. These data are obviously unreliable and are not presented. It seems probable that almost all univalents reach the plate and that a small percentage of nonlagging, nonsplitting ones do occur. The relative frequencies will be dealt with later.

Univalents

One of the chief features of the autotetraploid microsporocyte is the asynapsis. The chromosomes are associated in all possible combinations which do not exceed four members (in plants which are strictly euploid). This presumably occurs because each chromosome is to some extent impeded in its attempt to associate with a partner, and thus many chromosomes fail completely to pair. The exact nature of this inability invariably to form bivalents or quadrivalents is not now clear. Several explanations are conceivable. (*a*) The spatial relationships within the nucleus are so different from those in the diploid that the homologous chromosomes have longer and more difficult paths to traverse in order to pair; the diploid may be below this threshold of difficulty. (*b*) The chromosomes may be able to pair homologously so far as spatial considerations are concerned, but some may be so situated that the attractions of homology with two other threads are so evenly balanced that the chromosome may not move effectively in response to either homologous thread, and thus at least two univalents would occur. (*c*) It is also possible that many of the associations may be so incomplete and weak that univalents split off from them before the late diakinesis or early metaphase stages are reached. (*d*) The tetraploid condition may be one in which occur various physiological unbalances affecting many intracellular processes, and thus the effect on meiosis might be a general adverse effect on synapsis, which is a common result of the application of unusual external conditions (heat, cold, and reagents).

No information is now available which would indicate how any of these possibilities could be tested or how the observations on existing material could be planned to clarify the matter. The following data were secured from a random sample of fifty autotetraploid individuals, approximately half of which were grown in the field and half in the greenhouse, so that environmental effects could be noted. A careful arrangement of the data showed no differences between the two groups, therefore they are treated as a single group. The observations were made and tabulated in the following manner. Anthers which had a

high percentage of anaphases were reduced to a very fine suspension on the slide, which was covered, heated, and then flattened to a degree which placed all the anaphase chromosomes in approximately a single focus. If carefully done, this flattening spreads the chromosomes over an area larger than usual and increases the separation between the anaphase groups. The univalent chromosomes, if there are any, are left clear and unmistakable in the middle of the cell. In this section the term univalents will indicate univalents observed or counted at anaphase. It will be clear that these univalents are the only ones which can be counted with accuracy in usable frequencies. At diakinesis, and especially at metaphase, the univalents can occur in positions which cannot reliably be determined to signify association or asynapsis. The anaphase univalents seem to constitute most, if not practically all, of the univalents in the material. Those not passing to the plate will be considered later.

In the 5000 anaphase cells examined—100 cells from each of fifty plants chosen at random—the average number of univalents was 0.72 per cell. The range of the univalent frequency was relatively wide, extending from 0 to 8 univalents, an interval which includes approximately 29 per cent of all the chromosomes in the euploid cells. These fifty plants were made up of three groups which had different somatic chromosome numbers. Six plants had 29 chromosomes, four had 27, and forty had 28. These groups were kept separate, so that the effect of chromosome number on synapsis might be determined. The aneuploidy presumably might increase the chromosome unbalance and so affect the pairing. The six plants with 29 chromosomes had an average of 0.99 univalents per cell, and the four plants with 27 chromosomes had 0.70 univalents per cell. The euploid plants, those with 28 chromosomes, had 0.68 univalents per cell. The data are presented in table 1.

If the 28- and 29-chromosome groups are compared, it will be seen that the differences between them seem relatively large. Such a comparison was made on the basis least favorable for showing a difference. The groups were compared, not on the basis of the total number of univalents but with respect to the total number of cells with univalents. Table 1 indicates that the 29-chromosome group had more cells with higher univalent numbers, a fact which would increase the difference if it were based on total univalent number. The difference between the 28- and 29-chromosome groups, computed on the basis of cells with univalents, is 16 per cent and $X^2 = ca.\ 30$. It seems clear that the increase in asynapsis which occurs in individuals with $4n + 1$ chromosomes, when compared with that in 4n individuals, is greater than can be accounted for by assuming that the extra chromosome fails to pair. It seems clear that the extra chromosome in these six individuals introduced an unbalanced condition which impeded the pairing or induced precocious separation of the chromosomes. The data seem to indicate that the addition to the euploid number was more serious than subtraction from it, for the magnitude of difference between the 28- and 27-chromosome groups is not nearly so great. The difference between these two groups is 5.7 per cent and $X^2 = ca.\ 3.4$, a difference which may be presumed to be without significance in this material. These values indicate that in this autotetraploid the additional asynapsis induced by increasing the chromosome number by one is far greater than that induced by decreasing it an equal amount.

No morphological effects on the individuals with aneuploid numbers were observed. If the chromosome numbers of the fifty plants are considered, there is a slight indication from the numbers that 29-chromosome plants are more frequent than plants with 27 chromosomes. The counts of somatic chromosome numbers in a larger sample, however (table 2), indicate that no large difference, if any, exists.

TABLE 1

UNIVALENTS IN AUTOTETRAPLOID SECALE CEREALE

CHROMOSOME NO.	CELLS WITH UNIVALENTS								
	0	1	2	3	4	5	6	7	8
	Frequencies								
27	214	123	42	16	3	1	0	0	1
28	2326	959	493	151	53	8	4	3	3
29	253	183	109	39	9	5	1	0	1
Total	2793	1265	644	206	65	14	5	3	5
	Percentages								
27	53.50	30.75	10.50	4.00	0.75	0.25	0.00	0.00	0.25
28	58.15	23.98	12.33	3.77	1.33	.20	.10	.09	.09
29	42.17	30.50	18.17	6.50	1.50	.83	.17	.00	.17
Average	55.86	25.30	12.88	4.12	1.30	0.28	0.10	0.06	0.10

TABLE 2

DISTRIBUTION OF SOMATIC CHROMOSOME NUMBERS

Somatic chromosome no.	26	27	28	29	30	31
Frequency	0	9	82	9	1	0

It is of interest to consider meiosis in the individual with 30 chromosomes. If chromosome additions are deleterious at meiosis, this individual should have a more aberrant meiosis than the 29-chromosome plants. Meiosis in this plant was extremely irregular, asynapsis was most frequent, and fragmentation occurred in parts or all of most cells to a degree which made measuring the asynapsis impossible. Unfortunately no 26-chromosome plant was found, so the comparisons could not be extended to include deficiency as well as duplication for two whole chromosomes, with respect to the tetraploid euploid number. The observations on this 30-chromosome plant are merely suggestive, since the plant could have had some special constitution not frequent among the many other possible 30-chromosome combinations.

The hypothesis that the asynapsis is the result of homologous attractions so balanced among three chromosomes that the center one moves toward neither homologue but remains "suspended" between them can be investigated somewhat in these data. If such were frequently the case, then the asynapsis owing to all other reasons would have

superposed on it asynapsis for two and four chromosomes; that is, the classes with even numbers of chromosomes would be weighted to some degree by this "indecisive" homology. The actual data show no irregularities in the frequency distributions of numbers of cells and numbers of univalents (fig. 9). The absence of any tendency toward higher frequencies in the even-numbered classes seems clear from this graph. The regularity of the frequency distribution would seem to indicate that the asynapsis is a phenomenon which has a random basis and that this is related to the regression coefficient—0.40878 for the 28-chromosome plants. If the three groups are plotted separately, the regularity of the distributions is wholly consistent. The lines or curves have different slopes, but are equally without interruption in approaching the X axis.

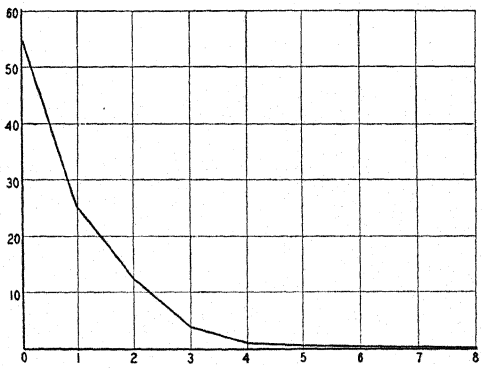


FIG. 9.—Percentages of cells (based on 5000) plotted against number of laggard univalents at anaphase I.

CHROMOSOME BRIDGES

The tetraploid microsporocyte differs from its ancestral diploid further in that its chromosomes possess structural alterations which become obvious at the first meiotic anaphases. An adequate examination, which included several thousand cells, indicated that the diploid had no such chromosome differences. The differences are those in which segments of the threads become rearranged or reduplicated so that bridges extend between bivalent halves at anaphase I. There are two simple ways in which these changes may be imagined to have occurred. The stresses within the tetraploid microsporocyte may be greater in some instances than the threads can withstand, and thus the threads may be ruptured and then reunite in a different order. The unusual homologous attraction among four chromosomes may sometimes compel non-homologous pairing, which is followed by crossing-over or some other break-producing mechanism. The reunion from the crossing-over or breaking could produce a different arrangement of chromosome segments and consequently bridges at meiosis in the next generation.

These bridges are numerous and of various types. The two most frequent may be seen in figures 2 and 8. The uppermost bridge in figure 2 is a simple one which extends from insertion point to insertion point. It has no chromosome fragment near it. The bridge in figure 8 is similar, except that it has a small fragment beside it. This bridge and fragment are simply explained by assuming an inversion within the chromosome arm of one homologue. The size of the inversion is indicated by the size of the acentric fragment. The inversion which produced the upper bridge in figure 2 is similarly explicable, except that it probably was produced by a three-strand double cross-over. Such a cross-over would leave no acentric fragment. It would require a much larger inversion than that in figure 8. The lower bridge in figure 2 is directly comparable with that in figure 8. Superficially this bridge seems to extend between three chromosome arms at one

pole and four at the other, an anomalous arrangement. But what appears to be a chromosome arm at the left of this bridge is probably a fragment, comparable in size with that of the inverted segment. Such fragments frequently pass toward one pole and are thus included in the telophase nucleus. It is clear that this inversion must have involved nearly a whole chromosome arm. These bridges can also be accounted for by assuming duplications instead of simple inversions, as has been done here.

A different kind of bridge may be seen in figure 4. This is stretched between two univalent halves. It can be explained simply by postulating a stickiness at the chromosome ends, such as is known to be present when ordinary bivalent bridges occur without apparent cause. It can also be accounted for on the basis of a rupture in the thread during diakinesis, when the chromosome may have been associated. It is not difficult to imagine that the forces which repel the pairs of threads may infrequently produce complete disjunction and rupture the threads in the unusual autotetraploid microsporocyte. According to McCLINTOCK (1), such a rupture would presumably fuse at the point of the break and thus produce a dicentric univalent chromosome. This would form a bridge at anaphase I and would break at telophase, when fusion would again occur. In maize (1) this breakage-fusion-bridge cycle persists through the gametophytic and endosperm divisions. The hypothesis that the original break occurs by chance at diakinesis is supported by the fact that these bridges were present in all plants in approximately the same very low frequency. If this bridge parallels the similar ones in maize, it would appear last in the division preceding zygosis, after which the chromosome would appear normal.

In figure 7 may be seen a bridge at second anaphase. This one could be produced in the breakage-fusion-bridge cycle started during the first anaphase by inversion bridges, or bridges similar to the univalent bridge just described.

CHROMOSOME LOSS

One of the more unusual phenomena in this autotetraploid is that of chromosome loss. By this is meant the absence of whole chromosomes from the microsporocyte at anaphase I. The loss may include one to many whole chromosomes. A cell with such loss may be seen in figure 6. This cell has 8 chromosomes at one pole, 7 at the other, and a univalent dividing on the plate. The example was taken from a plant with 28 somatic chromosomes.

The data on this kind of cell indicated that practically all the plants must have produced such cells. Of the fifty plants examined, thirty-three had cells deficient for one or more chromosomes. In most instances these were losses for more than 5 chromosomes. It seems probable that among the many hundreds of anthers not examined were patches of deficient cells. The data were secured from two or three anthers from each plant and consequently do not constitute a reliable measure of the actual frequency of such deficiency.

The samples did indicate that in many instances the losses occurred several cell generations before meiosis. The patches of deficient cells included approximately thirty cells in some instances, indicating that the original loss occurred five cell generations before meiosis. In other instances the deficient cells were in pairs, an indication that the loss occurred in the mitosis immediately preceding meiosis. No exact and critical data were secured on the earliest generation at which the loss occurred, because the loss itself

could have resulted in a chromosome unbalance with detrimental effects on mitosis. Furthermore, if this were not true, the technique used in preparing these slides (crushing the anther and its contents) would not be expected to keep the deficient patches intact, and thus a more recent origin than the actual one would be indicated by the cell count. It is also possible that the loss could be progressive—the original loss being followed by further ones. Some evidence for this was found in the preparations. In some islands of deficient tissue were found smaller islands of cells with more extreme deficiency. This is most easily explained by assuming a progressive loss. There was little uniformity in the magnitude of the losses. Table 3 presents data from various cells selected at random.

The losses did not seem to be extremely deleterious to mitosis, since the islands frequently included many cells. In general, however, the more extreme losses were present in the smaller islands. The most extreme losses would be expected to leave the cells with full haploid complements. Even this should be a very severe loss in a plant like rye, which is extremely heterozygous. The actual extreme cases, however, represent losses which leave the cells with less than the haploid complement. Many anaphase cells seemed to possess this excessive reduction, but they have not been tabulated, since the metaphases alone can be safely used in this connection. In all instances except one these were found as single cells. The metaphase configurations are presented in table 3. The single exceptional instance may be seen in figure 5. This shows two adjacent cells, each of which has an identical complement of one trivalent and three univalents, indicating that the original cell, one cell generation removed, was deficient for three whole chromosomes (about 43 per cent of its basic chromosome complement or 79 per cent of its original complement) and still managed to go through a complete mitosis. The two cells illustrated could be seen to have their chromosomes oriented for another division, as is clear from the trivalents, before they were flattened for photographic purposes.

TABLE 3

METAPHASE CONSTITUTIONS OF DEFICIENT CELLS 2n=28

Univalents	Bivalents	Trivalents	Deficiency	Percentage deficient
12	6	2	8	29
3	6	0	19	68
4*	2	0	22	79
5	5	0	18	64
12	2	0	14	50
5	2	0	21	75
2*	2	1	23	82
4	4	1	19	68
4	3	0	21	75
1	7	0	20	71
0*	6	0	22	79
1	5	1	21	75
3*	0	1	24	86
5	2	5	16	57
1 and 2 quadrivalents*	.....	.....	25	89

* Indicates hypoploid cells.

These cells with viable hypoploid complements resemble those found in *Tradescantia* (4), in which microspores deficient for three whole chromosomes developed to the metaphase stage of the first microspore division. This case in *Tradescantia*, and comparable ones in other material, were explained by assuming that gene products from normal contiguous cells diffused into the deficient cells and afforded enough substance to permit normal development. This seems to be the simplest explanation of viability with extreme deficiency, and it probably accounts for the tolerance of deficiency in female gametes, which are con-

tiguous to normal tissue, while male gametes are isolated by heavy walls. The two cells deficient for three chromosomes constitute an instance in which the diffused material was sufficient to make the cell normal enough to divide. The possibilities should be considered that all chromosomes are not equally necessary, however, and that the lack of certain ones may be partially compensated for by duplication of others. The three missing chromosomes may be constituted of much nonessential or inactive material, and the trivalent may replace much of what they contribute to the cell. It would therefore be unsafe to state that a full three-sevenths of the genes and their products are missing. That a great deficiency does exist, and that it is compensated for by the diffusion of gene products from adjacent and contiguous cells, seems reasonable.

ANAPHASE DISTRIBUTIONS

The chromosome behavior just described was such as would produce gametes with anomalous chromosome numbers. Another source of such irregular numbers is the division or disjunction mechanism itself. Any tendency which the spindle may have toward distributing metaphase chromosomes unequally at anaphase would produce microspores with different numbers. The mechanism is extremely precise in the diploid. It may be equally precise in the tetraploid, or it may be affected by chromosome unbalance in the tetraploid, or it may simply be altered in its precision by tetraploidy itself. The data necessary for considering this problem can be secured with reasonable exactness and adequacy. Their dependability is impaired somewhat by the facts that no estimate of the frequency of nonlagging univalents could be secured and that a trivalent can divide as equally as possible and still produce an unequal anaphase. The first source of error must be accepted; the second will compensate itself in many or most cells. The cells in which two or more trivalents disjoin so that one pole receives twice as many chromosomes as the other are those in which the anaphase counts will imply a degree of irregularity in the spindle which does not exist. From these considerations it would seem safe to state that the degree of spindle irregularity measured is the minimum irregularity, or an underestimated regularity.

TABLE 4

ANAPHASE DISTRIBUTION IN AUTOTETRAPLOID SECALE CEREALE

PLANT CHROMOSOME NO.	DEGREE OF IRREGULARITY				
	0	1	2	3	4
	Frequencies				
27	313	82	5	.....	.....
28	3138	821	38	2	1
29	491	101	7	1	.....
Total	3942	1004	50	3	1
	Percentages				
27	78.25	20.50	1.25	.....	.....
28	74.85	20.52	0.95	0.05	0.03
29	81.83	16.83	1.16	1.00	.....
Average	78.85	20.08	1.00	0.06	0.02

In summarizing the data (table 4), the following procedure has been used. If the anaphase chromosome numbers are equal in a 28-chromosome plant, or differ by one in a 27- or 29-chromosome plant, the division has been classified as 0 degrees irregular. A difference of one in the former class, or two in the latter classes, has been classified as 1 degree irregular. In

this manner the divisions have been classified up to 4 degrees irregular. Where univalents occurred, their number was subtracted from the total number and the irregularity then determined as before—by the degree it departed from the best possible disjunction. An inspection of the percentages (table 4) seems to indicate that the spindle mechanism must be extremely precise—perhaps as exact as it is in the diploid. The average percentage of cells with 1 degree of irregularity is 20 per cent. This whole class can be accounted for merely by assuming that in one cell in five a trivalent and its corresponding nonlagging univalent were unequally distributed and uncompensated by similar behavior in the opposite direction by another trivalent. This means that such behavior need occur but once in 140 chromosome associations, if no other source of irregularity exists. However, another source of irregularity does occur. Two nonlaggards at the same pole would produce an unequal result. It would seem that the spindle mechanism is perhaps as precise as that in the diploid, and that the irregular anaphases are largely the results of chromosome associations which can produce irregular results from regular disjunctions, that is, trivalents and nonlagging univalents. Since there are but 20 per cent irregular cells, and since this can be accounted for by assuming a very low percentage, 0.71 per cent, for random phenomena which are known to occur, it would seem more probable that the spindle mechanism is precise and that 0.71 per cent indicates the combined frequencies of nonlaggards at the same pole, trivalents disjoining in the same direction, and trivalents and nonlagging univalents producing 3/1 distributions.

The data in table 4 seem to indicate further that the group with 29 chromosomes had more regular anaphase distributions than had the other two groups. The X^2 for this class compared with the 28-chromosome class is *ca.* 3.8, or just below the 5 per cent value.

Another separation of the data, in which the number of univalents per cell is tabulated against the degrees of irregularity, is presented in table 5. The table

TABLE 5

ANAPHASE DISTRIBUTIONS IN AUTOTETRAPLOID SECALE CEREALE

No. of univalents in cell	Degree of irregularity				
	0	1	2	3	4
	Frequencies				
0	2171	596	25	1	.....
1	1064	188	11	0	2
2	469	165	9	1	.....
3	172	32	2	.....	.....
	Percentages				
0	77.73	21.34	0.89	0.04	.....
1	84.11	14.86	0.87	0.00	0.15
2	72.83	25.62	1.40	0.15	.....
3	83.50	15.53	0.97	.....	.....

indicates that the distributions are not correlated with univalent number.

SOURCES OF MICRONUCLEI

Recently the relation between anaphase laggards and tetrad micronuclei has received much attention (2). According to the scheme presented by other writers, each laggard splits at anaphase I, each half passing to its nearest pole. At anaphase II these halves are distributed at random to the poles. If these events occurred with regularity, deficient and duplicated quartet members would be formed but no micronuclei. But the half

univalents from the laggards at division I do not all reach the poles at division II soon enough to be included in the telophase nucleus, and thus micronuclei are formed. If the frequency of laggards is known, certain predictions about the distributions and frequencies of micronuclei can be made. Thus, one laggard should produce as a maximum two micronuclei, each of which must occur in a different microspore. Two laggards would produce four micronuclei, with two as the maximum in a microspore. The data secured in these observations indicate that no such regularity occurs, since micronuclei are found in unexpected frequencies and combinations. That many micronuclei are wholly unrelated to simple anaphase laggards may be seen in several of the photomicrographs included here. Figure 2 shows a division with one laggard which has split and is passing to the poles, while two bivalents are so delayed that the telophase nuclei will probably be formed before the bridges break and allow the bivalent halves to be wholly included. Figure 8 shows three laggards splitting, while a bivalent has an inversion or duplication bridge, the fragment of which will form a micronucleus. Figure 4 is of a univalent which has one set of ends joined, presumably from a previous rupturing of the ends, so that it may well split into three chromatin masses instead of two. Such breaks are not infrequent, wherein the two main masses of chromatin, the univalents, are toward the poles while the bridge remains in the center near the cell plate, and in some instances is divided in two by the plate. The data also indicate that, while in general the frequency of laggards observed at anaphase is distributed more or less in a regular fashion (that is, a distinct mode exists), it is also true that at both metaphase and anaphase exceptional cells occur which have an unexpected number of univalents and laggards. Such cells would produce tetrads with many more than the predicted numbers of micronuclei. Another source of anomalous tetrads may be metaphase cells with a reduced number of chromosomes. Such deficiency extends from losses of single ones to a loss of 22 chromosomes, or 79 per cent of the individual's initial complement. Although these cells usually divide normally at division I, they have a higher percentage of univalents than normal microsporocytes. Furthermore, while they do complete the first division normally, many of the nuclei which result from this division enter the second division with fewer than a normal haploid complement of chromosomes and may be presumed, in some instances at least, to yield an abnormal contribution to the tetrad members. There are therefore many ways in which micronuclei may be formed which are wholly or partially independent of the laggards which occur at anaphase I. This conclusion is supported by observations on the sizes of micronuclei, which range continuously from extremely small chromatin dots which are hardly visible to large reticulate masses which must include several chromosomes. In several instances single quartet members had two isodiametric nuclei. It seems clear that single anaphase laggards cannot vary in size from representative micronuclei down to the smallest ones, which have a volume less than one-tenth of average. It seems probable that any scheme for predicting the numbers and dispositions of tetrad micronuclei from the numbers of anaphase laggards can predict only the minimum numbers and simplest dispositions. In some instances, as in the present material, these predictions would have only a very approximate, if any, reliability.

Summary

1. Meiosis in autotetraploid *Secale cereale* is characterized by multivalent associations, inversion or duplication bridges, univalent bridges, univalent laggards and nonlaggards, irregular anaphase distributions, and chromosome loss.

2. The degree of anaphase irregularity is so low as to indicate that the spindle mechanism is as precise in the autotetraploid as in the diploid.

3. Unequal anaphases are produced by nonlagging univalents and coincidences in the divisions of trivalents.

4. Chromosome loss seems to interfere little with mitosis until the reduction leaves little more than the haploid number. Hypoploid cells were capable of division, the extreme instance being the division of a cell deficient for three whole chromosomes. The loss occurs frequently, usually within five or six cell generations before the first meiotic division, and may be followed by additional loss within the islands of deficient cells.

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LITERATURE CITED

1. McCLINTOCK, B., The stability of broken ends of chromosomes in *Zea mays*. Genetics **26**:234–282. 1941.
2. MYERS, W, M., and HILL, HELEN D., Studies of chromosomal association and behavior and occurrence of aneuploidy in autotetraploid grass species, orchard grass, tall oat grass, and crested wheat grass. BOT. GAZ. **102**:236–255. 1940.
3. SAX, KARL, Chromosome structure and the mechanism of crossing-over. Jour. Arn. Arb. **11**:193–220. 1930.
4. ———, Diffusion of gene products. Proc. Amer. Nat. Acad. Sci. **28**:303–306. 1942.

COMPOSITION OF THE TOMATO PLANT AS INFLUENCED BY NUTRIENT SUPPLY, IN RELATION TO FRUITING[1]

D. I. ARNON AND D. R. HOAGLAND

Introduction

This paper presents the results of some biochemical studies carried out in the last several years as part of an investigation of the relation of the nutrient medium to the growth and fruiting of the tomato plant. Incidental to large-scale experiments involving the use of artificial culture media and soils (3), an opportunity presented itself to explore the effects of different nutrient environments on the composition of fruit and foliage of plants maintained under the same climatic conditions. In addition, an attempt was made to relate the effects of fruit-bearing to the organic and inorganic composition of plants grown in complete as well as in potassium- and phosphorus-deficient nutrient solutions.

METHODS.—The plants were grown by the large-scale culture methods already described in detail (3). Unless otherwise noted, black iron tanks with a capacity of 460 liters were used in all nutrient-solution experiments. The soil and sand cultures referred to are those previously described (3). The methods of germinating seeds and the nutritional history of seedlings prior to transplanting to the experimental medium are—except for the stated differences—also the same as those given in the earlier publication.

The seven nutrient solutions used are presented in table 1. Iron was added once or—as the plants became larger—twice weekly at the rate of 0.66 cc. of 0.5 per cent iron tartrate or iron sulphate solution to a liter of nutrient solution.

TABLE 1

COMPOSITION OF NUTRIENT SOLUTIONS IN TERMS OF MOLAR CONCENTRATIONS OF COMPONENT SALTS*

SALT	NUTRIENT SOLUTIONS						
	I	II	III	IV	V	VI	VII
KNO_3	0.006	0.010	0.001	0.010	.....	.....	.....
K_2SO_4	.....	.....	.....	.....	0.005	.....	0.005
$NH_4H_2PO_4$	0.001	0.002	0.002	0.0002	0.002	0.002	.....
$(NH_4)_2SO_4$	.....	.....	.....	0.0009	.....	.....	0.001
$Ca(NO_3)_2$	0.004	0.003	0.0075	0.003	0.006	0.006	0.006
$MgSO_4$	0.002	0.002	0.002	0.002	0.002	0.002	0.002

* All solutions except nos. I and II received the supplementary micro-nutrient solution A_5 (2); solutions I and II received the supplements A_4 and B_7 (1), except that in the experiment represented in table 4 solution II received only the supplement A_5.

The standard procedures previously referred to (3) were used in analyzing the nutrient solutions and in determining the inorganic constituents of plants. Total nitrogen was determined by the Kjeldahl method.

For the analysis of organic constituents, the plants were boiled, immediately after harvesting, for 15–20 minutes in a suitable volume of 95 per cent ethyl alcohol, computed to give an effective concentration of 80 per cent alcohol after dilution by the plants. Extraction was completed in a Soxhlet apparatus. The extract was analyzed for total soluble ni-

[1] C. M. JOHNSON and W. E. FRATZKE have rendered valuable analytical assistance in the course of this study.

trogen by the reduced-iron modification of the Kjeldahl method; it was also analyzed for total and reducing sugars (5). The residue from the Soxhlet extraction was analyzed for starch (6) and for total nitrogen by the Kjeldahl method. The total nitrogen of the residue multiplied by the factor 6.25 is reported as protein nitrogen.

All analyses except those of the alcoholic extract were carried out on dried and ground material. Unless otherwise specified, each fruit sample was prepared for analysis by selecting from each treatment enough uniform fruits of the same degree of ripeness to give 1 kg. of fresh weight. Leaf samples were likewise prepared by pooling the detached leaves from each treatment and taking for analysis after thorough mixing a sample of about 100–200 gm. of fresh weight. The plant material was dried at 70° C. in a ventilated oven and was ground in a Wiley mill to pass a 60-mesh screen.

Results

Effect of nutrient media on inorganic[2] composition of fruit

Table 2 presents the results of analyses of tomato fruits harvested from plants grown side by side in the same greenhouse in soil, water, and sand culture. As shown by the previously reported (3) large yields from these cultures, all three media had an adequate supplying power for nutrients, and the plants were grown under climatic conditions conducive to fruitfulness. The data in table 2 indicate that in these circumstances the inorganic composition of fruit grown in the three different media was relatively constant, with the exception of magnesium. No marked variation was found in the water content of the three kinds of fruit grown in the three types of media.

[2] Inorganic composition and similar terms are here used with reference to elements introduced into the plant through absorption by roots, whether or not these elements subsequently enter into organic combinations.

Effect of total concentration and relative composition of nutrient solution on composition of fruit and foliage

The relation of the composition of the nutrient medium to that of the plant was further explored, with the aid of the nutrient solution method, which easily lends itself to controlled variation of the external supply of nutrients. The influence of total concentration of the nutrient medium was followed in an experiment, the results of which are presented in table 3. The plants, after having been grown for several weeks in a basic nutrient solution (solution II, table 1), were divided at the onset of the first flower clusters into three groups of twenty plants each and transferred into three nutrient solutions. The first group of plants continued to grow and set fruit in a basic nutrient solution of the same composition as the original one; the second group was placed in a solution of twice, and the third group in a solution

TABLE 2

Effect of nutrient media on composition of greenhouse tomatoes, variety Lloyd Forcing (p.p.m. of fresh weight)

	Water culture*	Sand culture*	Soil
K	2238	2175	2085
P	321	352	239
Ca	99	94	104
Mg	69	54	86
N	1270	1230	1090
Moisture (%)	95.3	95.9	95.1

* Solution I (table 1) used as source of nutrients.

of three times, the concentration of the basic nutrient solution.

Analysis of the ripe fruit from the three treatments (table 3) showed that, despite the differences in the total concentration of the external nutrient medium, the inorganic composition of the fruit remained essentially unaffected. There was no distinct relation between the water content of the fruit and the total concentration of salts in the external medium.

TABLE 3

EFFECT OF CONCENTRATION OF NUTRIENT SOLUTION ON COMPOSITION OF GREENHOUSE TOMATOES, VARIETY SUTTON'S BEST OF ALL (P.P.M. OF FRESH WEIGHT)

	Basic*	Twice basic concentration	Thrice basic concentration
K	2950	3040	2780
P	380	369	379
Ca	34	49	42
Mg	105	106	99
N	1652	1515	1690
Moisture (%)	95.7	94.6	94.9

* Nutrient solution II (table 1).

This relative constancy of the composition of the fruit is also apparent from the data given in table 4. In this experiment the plants were grown in three solutions of approximately the same total concentration but differing in their supply of potassium and phosphate ions. In the control solution the potassium concentration was allowed to fluctuate between 400 and 200 p.p.m. and that of phosphate between 200 and 100 p.p.m. In the low-potassium solution the potassium concentration was maintained between 40 and 0 p.p.m.; the solution was not allowed to remain without potassium for any appreciable length of time, and on approaching the zero point it was restored to a concentration of 40 p.p.m. In the low-phosphate treatment the phosphate concentration was maintained between 20 and 0 p.p.m. but was never allowed to remain at zero. Thus the low-potassium and low-phosphate treatments are to be regarded, not as deficient cultures but as nutrient media which have been supplying potassium and phosphate, respectively, from a low concentration. All three solutions were analyzed at frequent intervals, and salts were added as required.

TABLE 4

EFFECT OF LOW K AND PO_4 ON MINERAL AND MOISTURE CONTENT OF TOMATO FRUIT AND FOLIAGE, VARIETY SUTTON'S BEST OF ALL (P.P.M. OF FRESH WEIGHT)

	Fruit			Leaves		
	Control*	Low K	Low PO_4	Control*	Low K	Low PO_4
K	2650	2230	2490	5120	3900	5300
P	333	333	350	1318	1321	646
Ca	42	51	39	2770	3500	2320
Mg	105	88	94	1060	730	800
N	1530	1460	1480	5320	5610	5240
Moisture (%)	94.9	95.3	95.0	87.9	87.8	88.3

* Control treatment received nutrient solution II; low K and low P received solutions III and IV, respectively (table 1).

The effect of the different levels of external supply of potassium and phosphate on the composition of the fruit was contrasted with that on the composition of the foliage. Mature but not senescent leaves were picked, generally from about 1–3 feet from the tip of the plant. The plants were trained to a single stem and allowed to extend vertically up to a height of 10–12 feet. No distinction was made in this analysis between leaf blades and petioles.

As shown in table 4, the low phosphate supply in the nutrient medium was associated with marked reduction (approximately half) of the phosphorus content of the leaves, while that of the fruit remained about the same as in the control.

Despite the known interrelations between nitrate and phosphate absorption, the nitrogen content of the low-phosphate leaves remained essentially unchanged. This can probably be attributed to the very liberal supply of nitrogen in all the cultures. As to the potassium content of the plant, both leaves and fruit showed a reduction in the low-potassium treatment as compared with the control, but the reduction was more marked in the leaves than in the fruit. The fruit from the low-potassium treatment was characterized by softening on the vine before attaining full color. It is possible that the small decrease in the potassium content as a result of a low external supply is associated directly or indirectly with firmness of the maturing fruit. On the other hand, the larger differential of potassium content in the leaves may have been related to modified production of organic metabolites in the leaf, which influenced the character of the fruit.

The data in table 4 invite comparison as to the relative proportions of the inorganic constituents of leaves and fruit. The outstanding difference lies in the markedly lower concentration in the fruit of calcium and magnesium in relation to potassium. These and subsequent data (table 5) indicate that this relative proportion of bases is on the whole a fixed characteristic of the fruit, and it does not appear to be readily influenced by fluctuations, short of total deficiencies, in the external supply of nutrients.

The mineral composition of the fruit was modified in essential respects only when the plants were grown, for the period subsequent to flowering, in a nutrient medium totally deficient in some nutrient element. Table 5 compares the analyses of fruits, both young (green) and mature, grown in nutrient solutions deficient either in potassium or in phosphate, with fruits grown simultaneously in a complete solution. The number of fruits from each treatment included in the analysis varied from nine to eighteen for the ripe and from sixteen to fifty-nine for the green fruit. Each analytical value in table 5 represents the average of two independent but closely agreeing determinations made on samples secured from parallel cultures. The conditions under which the plants were grown will be discussed in the following section, and it will suffice to state at this point that the plants, later identified as fruitful in the defloration experiment, were kept in the respective media from the onset of the first flower cluster. Corresponding data for the composition of the foliage will be found in figures 4, 5, and 7.

TABLE 5

EFFECT OF POTASSIUM AND PHOSPHORUS DEFICIENCY ON MINERAL AND MOISTURE CONTENT OF TOMATO FRUIT, VARIETY MARGLOBE (P.P.M. OF FRESH WEIGHT)

	Ripe			Green		
	Complete	Minus K	Minus PO_4	Control	Minus K	Minus PO_4
K	2645	1150	2255	2395	1420	2210
P	529	570	116	473	707	112
Ca	42	60	46	34	130	51
Mg	106	94	130	118	123	147
N	1762	2660	1510	1417	2930	1385
Moisture (%)	94.3	93.8	95.5	94.4	93.4	96.5

Of course, the "ripe" fruit produced by the potassium- and phosphorus-deficient plants was in no sense of marketable quality. The fruit was of small size, poorly developed, and in the minus-phosphate cultures it consistently exhibited symptoms closely reminiscent of blossom-end rot. As the subsequent discussion will show, the potassium and phosphorus of the fruit in the deficient cultures was derived from the reserves of these elements in the foliage, absorbed

during the preliminary period when all plants were grown in a complete nutrient medium. The great differences in the mineral content of the fruit shown in table 5 cannot therefore be taken as indicative of the normal range of variation, but rather of redistribution of the potassium and phosphorus reserves within a plant in which the development of the fruit has been initiated before the external supply of nutrients has become deficient. The previously stated conclusion about the relative constancy of the mineral composition of normal fruit remains unaffected by the present data.

Table 5 shows that the fruit from the minus-phosphate cultures had more than a fourfold decrease in its phosphorus content as compared with the fruit from the complete nutrient solution. Likewise, the potassium content of the tomatoes from the minus-potassium treatment was less than half that in the normal fruit. These differences in composition were apparent not only in the ripe (fully-colored) but also in the immature green fruit. (This included assorted sizes, however, and cannot be regarded as physiologically uniform.) A high calcium content was found in the potassium-deficient fruit, particularly in the young fruit. It seems reasonable to expect, from the known interrelations of cations, increased calcium migration to the potassium-deficient fruit, especially to the young developing fruit, which has a high capacity for solute absorption. The potassium-deficient fruit showed, particularly when the results were expressed on a fresh-weight basis, an unusually high nitrogen content.

The subsequent increase in the size of the green fruit, accomplished largely by the enlargement of the size of the existing cells and accompanied by the intake of water, would explain the decrease of calcium on a concentration basis in the ripe fruit. The higher calcium content in the potassium-deficient plants was not confined to the fruit but was also evident in the foliage (figs. 4, 5). This points to an increased calcium absorption (per unit weight of plant) from the nutrient substrate by the plants grown in the potassium-deficient solutions. The phosphorus-deficient had a higher magnesium content than the normal fruit.

Defloration experiments

The contrast between the composition of fruit and foliage has suggested the desirability of investigating the effect of fruit-bearing on the inorganic nutrient distribution in the plant. This was studied under three conditions of external nutrient supply: complete, minus-potassium, and minus-phosphate nutrient solutions (V, VI, and VII, respectively, table 1). With the exception of the deficient nutrient, the concentration of all the other elements, save sulphur, was the same in the three solutions. The sulphate ion was used in balancing the ionic charges.

Tomato plants of the Sutton's Best of All and Marglobe varieties were germinated in sand and grown in a complete nutrient solution (half strength of solution II, table 1) for a period of about 5 weeks, until the first flower clusters emerged. The plants were then selected for uniformity and divided into three groups of twenty-four plants each. Each group of twenty-four constituted one nutrient treatment, which comprised four metal tanks of solution, each tank having a capacity of 115 liters and supporting six plants. Within each treatment in turn, half the plants, that is, those in two tanks, had their flowers removed as soon as they emerged. The plants in the remaining two tanks were allowed to set

fruit.[3] The flowers on the fruitful plants were tapped regularly to insure pollination.

EFFECTS IN COMPLETE NUTRIENT SOLUTION.—As shown in figure 1, the fruitful plants in the complete nutrient made excellent growth and produced a relatively heavy crop of fruit. Unusual growth effects, however, were observed in the deflowered series. After several weeks the stems, petioles, and leaves became much thickened, rootlike structures appeared on the stems, and new growing points emerged prolifically from the leaf axils (fig. 2). These growing points were soon removed, together with all side branches, thus confining growth to the main stem. The characteristic thickening of the stem tapered down to the terminal growing point, which withered and died (fig. 3). These symptoms were reproduced in a second experiment carried out in a different season.

The profound morphological changes as a result of defloration were associated with a change in the organic composition of the plant (table 6). Samples of leaves were taken from the deflowered and fruitful plants at the conclusion of the experiment, 93 days after defloration. They consisted of the second and third leaves below the tip of each plant. The leaf blades and the petioles (which included the midribs) were analyzed separately. In addition, analyses of stems were made, for which the terminal 2-feet sections were used. Samples from plants grown in one tank were analyzed collectively, and since each deflowered and fruitful series comprised two tanks, this provided a replication of the analyses, which gave close agreement.

As shown in table 6, the differences in composition between the deflowered and fruitful plants were particularly notable in the stems and petioles. Common to all tissues from the deflowered plants was the marked accumulation of starch, especially pronounced in the stems,[4] in which

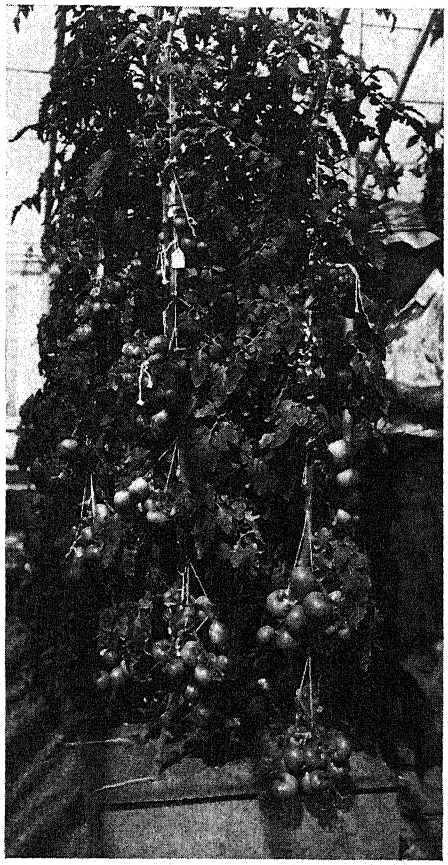
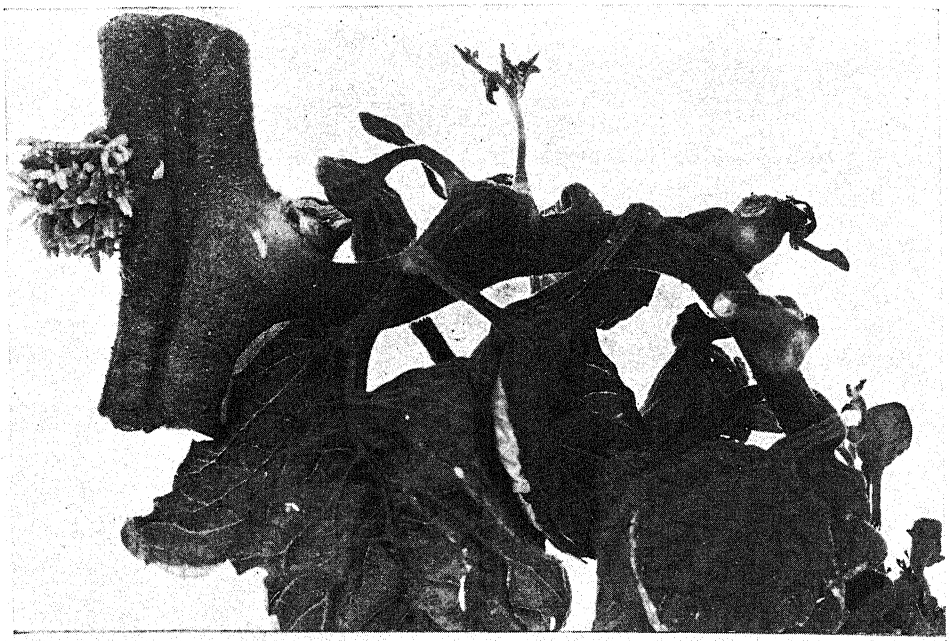


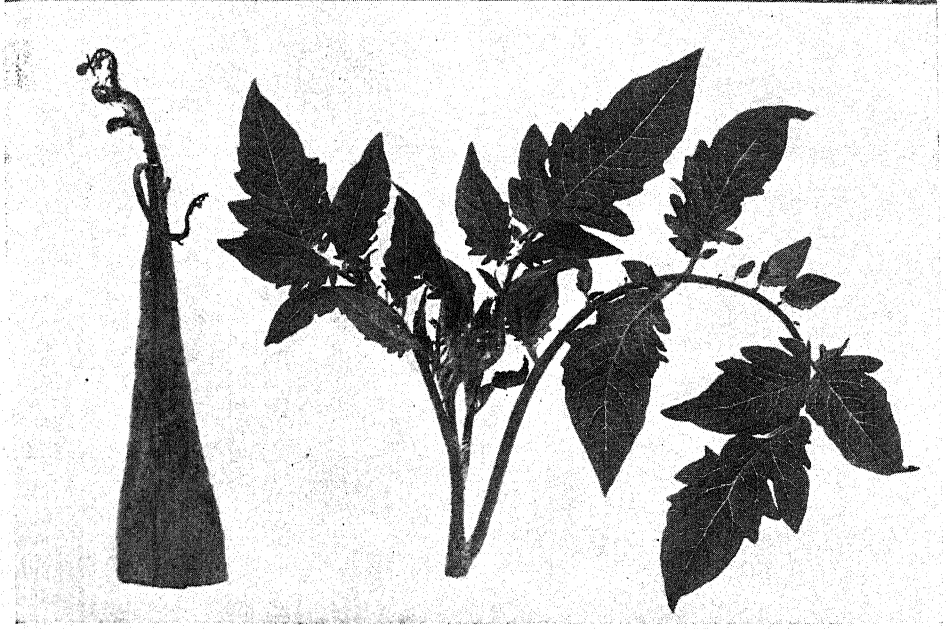
FIG. 1.—Fruitful plants in complete nutrient solution.

a sevenfold increase over the fruitful plants was found. It is to be noted that starch was accumulated under climatic conditions favoring growth and in the absence of any nutrient deficiency. The petioles and the stems of the deflowered plants also had a higher concentration of total sugars and of total soluble as well

[3] The former will be referred to as deflowered and the latter as fruitful.

[4] Confirmed in histological examination of the tissue by Dr. CHARLES HEIMSCH.





FIGS. 2, 3.—Fig. 2 (above), section of stem and leaf from deflowered plant grown in complete nutrient solution; note thickening and emergence of rootlike structures from stem and new growth at leaf axils. Fig. 3 (below), terminal growing point in deflowered (left) and fruitful (right) plant, grown in complete solution.

as protein nitrogen. Only negligible amounts of nitrate nitrogen were found in the alcoholic extracts of either fruitful or deflowered plants. In view of its continuous supply in the nutrient medium, the absence of nitrate in the tissues points to its prompt utilization—a course of events to be expected from the favorable supply of carbohydrates. Except for starch, the leaf blades of the deflowered plants showed no marked differences from those of the fruitful plants in the organic constituents for which analyses were carried out. Unlike the stems and petioles, the protein content of the "fruitful" leaf blades was found to be higher than that of the "deflowered" ones. The deflowered plants made more vegetative growth than the fruitful ones (table 7).

TABLE 6

ORGANIC CONSTITUENTS OF DEFLOWERED (D) AND FRUITFUL (F) TOMATO PLANTS GROWN IN COMPLETE NUTRIENT SOLUTIONS (PERCENTAGE FRESH WEIGHT)

	Leaf Blades		Petioles		Stems	
	D	F	D	F	D	F
Starch	1.91	1.35	0.35	0.13	1.05	0.15
Total sugars	2.19	2.05	2.67	1.81	3.31	2.95
Reducing sugars	2.01	1.92	2.08	1.72	2.51	2.65
Soluble N*	0.056	0.047	0.082	0.034	0.148	0.037
Protein N	1.93	2.51	0.89	0.71	1.05	0.69

* Only negligible amounts of nitrate nitrogen present.

Earlier in the growing period, 46 days after defloration, samples of leaves were taken for the determination of potassium, calcium, magnesium, and phosphorus. The deflowered plants exhibited at that time the already-described characteristic morphological symptoms but in less extreme form; the withering and dying of the terminal growing points was only at its inception. The leaves were collected in descending order from the tip of each plant and designated by ascendingly consecutive numbers. Leaf number 1 represented the first and number 6 represented the sixth consecutive leaf below the growing point. This method of sampling allowed the tracing of the gradient distribution of potassium, calcium, magnesium, and phosphorus in the plant from the youngest to the mature leaves. Leaves occupying the same position relative to the tip were pooled and analyzed collectively. Separate analyses were made on the leaf blades and petioles, with the latter including the midribs.

TABLE 7

AVERAGE FRESH WEIGHT OF SHOOTS AND ROOTS AT END OF EXPERIMENT (GRAMS)

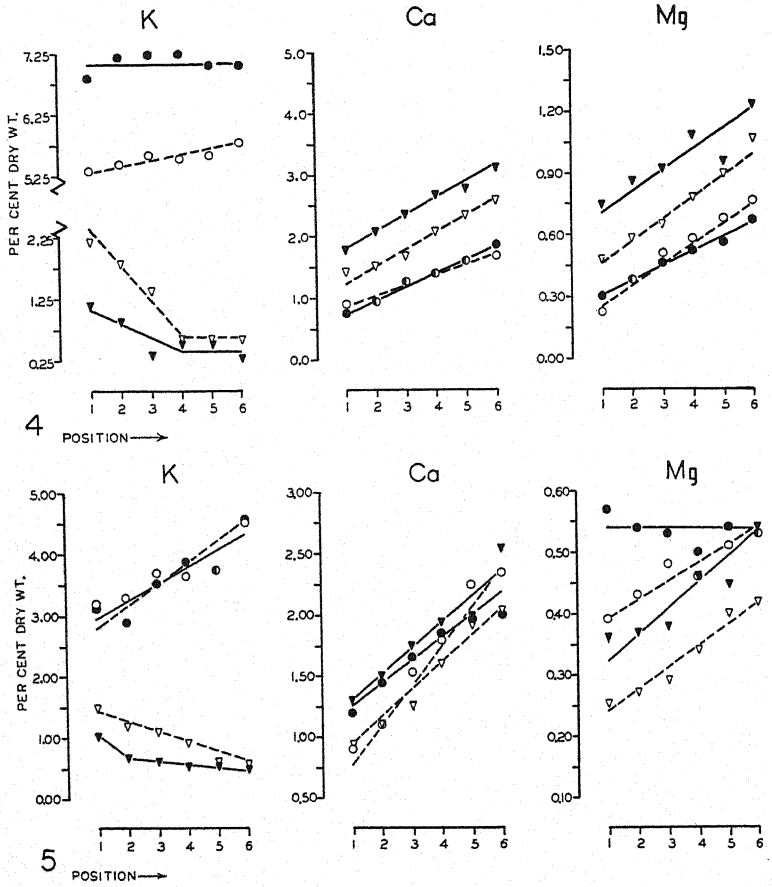
Treatment	Shoots*	Roots
Complete		
Fruitful	1570	339
Deflowered	2160	546
Minus K		
Fruitful	332	171
Deflowered	393	264
Minus PO_4		
Fruitful	302	178
Deflowered	530	259

* Weight of shoots is exclusive of fruits.

The results of potassium, calcium, and magnesium analyses are shown in figures 4 and 5. No outstanding differences are noted between the compositions of the fruitful and deflowered plants, except that the petioles from the latter had a lower potassium content than those from the former. This may possibly be an indication of active transport of potassium to the fruit, which—as shown previously—has a high requirement for this nutrient element. Close similarity was also found in the phosphate contents of the fruitful and deflowered plants (fig. 7).

EFFECTS IN MINUS-POTASSIUM NUTRIENT SOLUTION.—The removal of flowers from plants grown in the potassium-defi-

cient nutrient had a distinct effect on the alleviation of the potassium-deficiency symptoms. It is generally known that plants suffering from potassium deficiency exhibit a breakdown of the older leaves accompanied by a more or less normal formation of the youngest leaves, near the growing point, which withdraw the potassium from the older leaves. As the deficiency advances, the symptoms appear in progressively younger leaves, and ultimately, of course, the entire plant dies. While in the fruitful plants potassium-deficiency symptoms were evident near the tip, the deflowered plants made more growth (table 7), and leaves of a corresponding position on the vine appeared more healthy (fig. 6), showing less advanced potassium-deficiency symptoms in a deflowered plant on a leaf from the same position relative to the tip than on the leaf from the fruitful plant. Figure 6 also shows that the unusual growth proliferations noted on the leaves of the deflowered plants grown in complete nutrient (fig. 2) were much less pronounced



FIGS. 4, 5.—Fig. 4, petioles; fig. 5, leaf blades: potassium, calcium, and magnesium contents from fruitful and deflowered plants. Position numbers denote descending order of leaves from tip. Full triangles: fruitful, minus K. Open triangles: deflowered, minus K. Full circles: fruitful, complete. Open circles: deflowered, complete.

in the potassium-deficient deflowered plants.

Leaf samples for inorganic analysis were taken from the potassium-deficient plants, beginning near the growing point and progressing down, as previously described. The difference in the relative severity of potassium-deficiency symptoms between the fruitful and deflowered plants was reflected in the appearance of the collected leaves. In all twelve deflowered plants, the first and the second leaves (progressing from the growing point down) were free from deficiency symptoms, which were observed on leaves of similar position in some of the fruitful plants. The third leaf was free from deficiency symptoms in nine out of twelve deflowered plants but in only one out of twelve fruitful plants. The fourth, fifth, and sixth leaves were damaged in both the fruitful and deflowered plants (except for one healthy fourth leaf in a deflowered plant).

These symptomalogical differences are in accord with the analytical data presented in figures 4 and 5. The potassium content of the youngest leaves is distinctly higher in the deflowered plants, but in lower (older) leaves it approaches the level found in the fruitful plants. A reversal was noted in the case of calcium and magnesium, which were higher in the fruitful plants than in the deflowered ones. The lower potassium content of the fruitful plants is no doubt

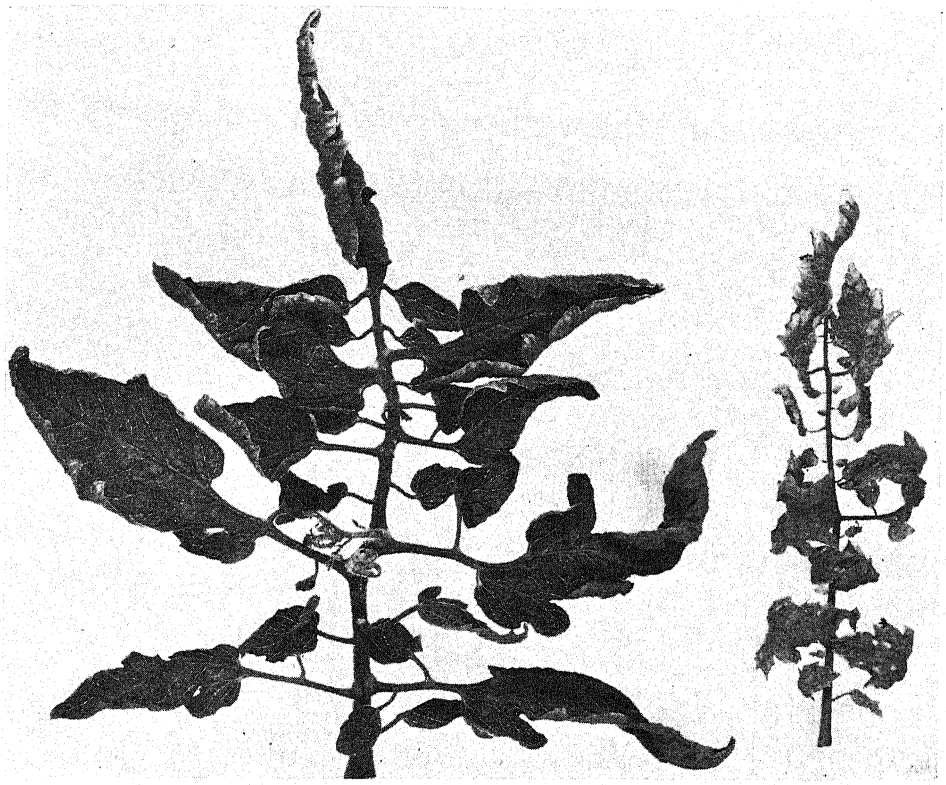


FIG. 6.—Effect of defloration on onset of potassium-deficiency symptoms in plants grown in potassium-deficient medium. Left, leaf from deflowered plant; right, leaf of corresponding position from fruitful plant.

attributable to removal of part of the potassium reserves to the developing fruit. The higher calcium and magnesium contents of the fruitful plants may be due to increased absorption of these cations to compensate for the export of potassium to the fruit. Under these abnormal conditions, calcium and magnesium con-

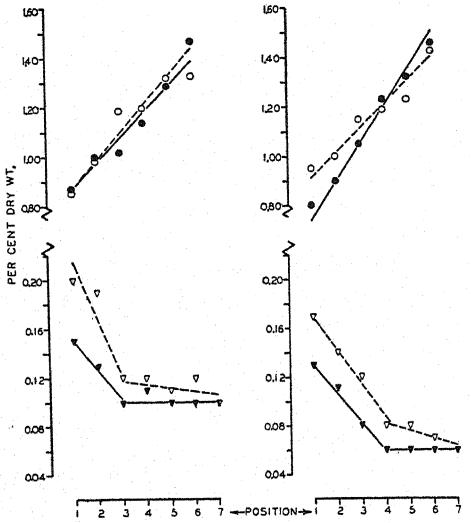


FIG. 7.—Phosphorus content of leaf blades and petioles from fruitful and deflowered plants. Upper and lower left, blades; upper and lower right, petioles. Position numbers denote descending order of leaves from tip. Full triangles: fruitful, minus P. Open triangles: deflowered, minus P. Full circles: fruitful, complete. Open circles: deflowered, complete.

ceivably may have replaced up to a certain point the function of potassium in balancing the organic acids in the leaves. In the absence of knowledge of changes in total calcium and magnesium in the leaves, these views are of course speculative. The data available pertain to percentage values on a dry-weight basis.

Figures 4 and 6 indicate differences in the potassium content of plants grown in complete and potassium-deficient nutrient solution. The former had several times more potassium, both in the fruitful and deflowered plants, than the latter. Moreover, with an abundant external supply of potassium, its concentration in the leaf seemed to increase in the older leaves, whereas the reverse was true in the potassium-deficient plants. As for the calcium and magnesium content, the plants grown in complete nutrient, unlike the potassium-deficient plants, showed no marked difference between the fruitful and deflowered plants.

EFFECTS IN MINUS-PHOSPHATE NUTRIENT SOLUTION.—Defloration of plants grown in a minus-phosphate nutrient solution produced effects on growth and composition similar to those reported for the potassium-deficient plants. The deflowered plants made more vegetative growth (table 7). Phosphorus-deficiency symptoms were more pronounced and more frequently encountered in the fruitful plants for any given leaf position on the vine. The results of phosphorus analyses (fig. 7) show a higher content in the youngest leaves of the deflowered plants than in the fruitful ones. These findings are in harmony with the evidence obtained by means of a radioactive isotope technique, that in the absence of adequate supply of phosphorus for both fruit and foliage the developing fruit draws on the phosphorus present in the leaves (4).

The phosphorus content of the leaves from the control plants, whether deflowered or fruitful, was several times higher than that for the phosphate-deficient plants (fig. 7). Defloration of plants grown in complete nutrient did not markedly affect the phosphorus content of the leaves, at least after several weeks. It will also be recalled that, unlike the deflowered potassium-deficient plants, defloration of the plants grown in complete nutrient did not greatly alter the composition of leaves with respect to inorganic

bases. These facts seem to warrant the conclusion that prevention of fruiting was important in modifying the inorganic content of vegetative tissues only when the external supply of nutrients was limiting. The marked differences found in the composition of leaves from potassium- and phosphate-deficient plants, including the reversal of concentration gradients between the old and young leaves, offer another line of evidence for the physiological validity of establishing potassium and phosphate deficiencies by methods of foliar diagnosis.

Discussion

The analytical data offer ample evidence that the inorganic composition of tomato fruit, as contrasted with the modifiable composition of the foliage, is relatively constant and not easily influenced by fluctuations in the external supply of nutrients, provided that outright deficiencies are absent. On a percentage weight basis the concentration of each of the inorganic nutrients studied is markedly lower in the fruit than in the leaves, but even more striking are the changes in the relative proportions of these constituents in the fruit. Potassium is the dominant element in fruit and—together with nitrogen—one of the two preponderant ones in the leaves. But the ratio of calcium:potassium shifts from approximately 1:2 in the leaves to 1:60 in the fruit and that of magnesium:potassium from 1:5 to 1:25 (computations based on data from the control treatment, table 4). The shifts in the proportions of phosphorus and nitrogen relative to potassium are less drastic but still appreciable. Another contrast with the leaf is the higher magnesium than calcium content of the fruit (on a percentage weight basis). The relative immobility within the plant of calcium as compared with magnesium and other mineral elements will be recalled in this connection (11). Of course, the figures cited apply only to a particular variety of plant grown under a given set of climatic conditions, yet other unpublished data indicate analogous relationships in other tomato varieties grown at various seasons.

A total deficiency, however, of either potassium or phosphorus in the nutrient medium, as contrasted with fluctuations above a minimum supply, is reflected in a decrease of these elements in the fruit, associated with a general growth disturbance and production of abnormal fruit (9). The results of the defloration experiments in the deficient cultures suggest that the developing fruit draws on the phosphorus and potassium reserves of the leaves, which leads to an accelerated breakdown of vegetative tissues. This is consistent with the general view that the regions undergoing most active growth and development, whether vegetative or reproductive, have the highest capacity for absorption and accumulation of inorganic nutrients, including the power of removal from physiologically less active plant organs.

The data, however, are incapable of establishing the mechanism by which the export of nutrients from older leaves in deficient plants to the developing fruit and younger leaves is accomplished. Two general types of mechanisms may be envisaged: one residing in the young tissue, which brings about the withdrawal of potassium or phosphorus from the older tissue through physiological changes in the latter incited by some hormone-like substance; another involving continuous circulation in the phloem of phloem-mobile nutrients (11). The second mechanism would require the further assumption that, in case of deficiency, the youngest tissues have the greatest power of reten-

tion from the phloem system of nutrients, such as potassium and phosphorus.

When a potassium- or phosphorus-deficient plant is deflowered, a potential sink for these elements is removed, and this is reflected in the higher concentration of these elements in the leaves. It is significant that the data in figures 6 and 7 indicate that the developing fruit is able to effect a decrease in the potassium and phosphorus content of even the youngest leaves. The mineral requirements of the developing fruit are satisfied at the expense of the vegetative tissues, if the external supply falls short of satisfying the needs of both (4). A comparison is suggested with the die-back condition of the prune, in which a severe leaf scorch is associated with low potassium content of the leaves (at least late in the summer) and a heavy crop of fruit. The condition was prevented by removing the crop by hand early in the season (8).

Defloration of plants grown in a complete nutrient solution did not result, within the sampling period, in marked increase of potassium, calcium, magnesium, or phosphorus in the vegetative tissues. It is possible that defloration, by removing a physiological sink, has impeded salt absorption in a manner analogous to the decreased salt absorption observed in "high-salt" plants (7). The suggestion is that the morphological effects of defloration (figs. 2, 3, 6) may be primarily associated with shifts in the organic constituents.

Attention has already been drawn to the accumulation of starch, sugars, and organic nitrogen in the deflowered plants. It is probable, of course, that even more striking changes were brought about by defloration in some other organic constituents not included in this investigation. Organic acids, and particularly growth substances or florigens, would seem to offer likely subjects for study. The unusual growth proliferation on the leaves and the occurrence of rootlike structures on the deflowered stems resemble those frequently produced by the application of hormone-like substances. The withering of the terminal growing point on the deflowered plants points to the likelihood of some toxic accumulation in this region of one or more organic constituents.

The morphological observations and chemical data leave no doubt that flowering and fruit development in a healthy tomato plant is a normal activity, which, if not permitted to proceed, results in important metabolic derangements. The unusual growth effects found on the deflowered plants may perhaps be regarded as an attempt on the part of the plants to make use, under the stimulus of growth substances, of the accumulated organic reserves which would normally lead to flowering and fruiting. As mentioned previously, the other outlet initiated by the plant for the utilization of these reserves, as reflected in the emergence of new growing points from the leaf axils, was also blocked by manual removal of the new growth.

The evidence here presented offers little encouragement to attempts to improve, from the standpoint of human nutrition, the mineral composition, with respect to calcium, of tomato or probably of other fruits by liberal soil fertilization measures. The tomato is not a rich source of calcium in the human diet, and even if marked increase in the calcium content of the fruit could be effected, it would not alter our dependence on other important sources of calcium, especially milk and cheese.

The prevalence of symptoms resembling blossom-end rot in the fruit from the phosphorus-deficient plants adds another observation to the etiology of this

disturbance. It seems possible that blossom-end rot may be associated with a number of nutritional causes all resulting in withdrawal of water from the blossom end of the fruit and subsequent breakdown of tissues.

The influence of nutrient conditions on the storage quality of tomatoes was investigated by MACGILLIVRAY (10).

Summary

1. Tomato plants were grown through the fruiting stage under greenhouse conditions in soil, sand, and large-scale water cultures, with nutrient solutions of varying composition.

2. The composition of the fruit with respect to constituents derived through absorption by roots was, in contrast to the modifiable composition of foliage, on the whole little affected by the kind of nutrient medium in which the plants grew. The composition was also not found to be materially altered in water-culture experiments in which the total concentration and relative proportions of nutrients were varied. Marked reduction was found in the potassium or phosphorus content of fruit developed on plants grown in a potassium- or phosphorus-deficient nutrient solution.

3. On a percentage dry-weight basis the concentration of potassium, calcium, magnesium, phosphorus, and nitrogen was markedly lower in the fruit than in the leaf, and the relative proportions of these nutrients were strikingly altered.

4. The manual removal of flowers in plants grown in a complete nutrient solution resulted in distinct alterations in growth, shown by thickening of stems and leaves, proliferation of new growing points at the leaf axils, and emergence on the stem of rootlike structures. Defloration was found to be associated with marked accumulation of starch in all tissues and of sugars and organic nitrogen in the stems and petioles. However, defloration was not found to influence materially the composition of foliage with respect to phosphorus, calcium, or magnesium. The potassium content of the petioles was higher in the fruitful plants, but no marked difference was found in the potassium content of leaf blades.

5. Defloration of plants kept in a potassium-deficient nutrient solution delayed the onset of potassium-deficiency symptoms and was associated with a higher potassium content in the young leaves of the deflowered than in the fruitful plants. The otherwise low potassium content of leaves showed an increasing upward gradient as contrasted with a downward gradient in plants grown in a complete nutrient solution. Downward calcium and magnesium gradients found in plants grown in complete nutrient were also found in leaves of potassium-deficient plants, but the latter had a higher content of these elements in the fruitful than in the deflowered plants.

6. Defloration of plants kept in a phosphorus-deficient nutrient solution delayed the onset of phosphorus-deficiency symptoms and was associated with a higher phosphorus content in the young leaves of the deflowered plants. The otherwise low phosphorus content of leaves from all phosphorus-deficient plants, whether fruitful or deflowered, showed—in contrast with the control plants—increasing upward gradient.

7. Several general considerations are given on the relation of the external nutrient supply and fruiting to the distribution of nutrients within the plant.

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LITERATURE CITED

1. ARNON, D. I., Microelements in culture-solution experiments with higher plants. Amer. Jour. Bot. 25:322–325. 1938.
2. ———, Vitamin B_1 in relation to the growth of green plants. Science 92:264–266. 1940.
3. ARNON, D. I., and HOAGLAND, D. R., Crop production in artificial culture solutions and in soils with special reference to factors influencing yields and absorption of inorganic nutrients. Soil Sci. 50:463–485. 1940.
4. ARNON, D. I., STOUT, P. R., and SIPOS, F., Radioactive phosphorus as an indicator of phosphorus absorption of tomato fruits at various stages of development. Amer. Jour. Bot. 27: 791–798. 1940.
5. HASSID, W. Z., Determination of sugars in plants. Ind. Eng. Chem. Anal. Ed. 9:228–229. 1937.
6. HASSID, W. Z., MCCREADY, R. M., and ROSENFELS, R. S., Determination of starch in plants. Ind. Eng. Chem. Anal. Ed. 12:142–144. 1940.
7. HOAGLAND, D. R., and BROYER, T. C., General nature of the process of salt accumulation by roots in the description of experimental methods. Plant Physiol. 11:471–507. 1936.
8. LILLELAND, O., Experiments in potassium and phosphorus deficiencies with fruit trees in the field. Proc. Amer. Soc. Hort. Sci. 29:272–276. 1932.
9. MACGILLIVRAY, J. H., Effect of phosphorus on the composition of the tomato fruit. Jour. Agr. Res. 34:97–127. 1927.
10. ———, Effect of nutrient root media on loss in weight and amount of rot in stored tomatoes. Proc. Amer. Soc. Hort. Sci. 41:289–292. 1942.
11. MASON, T. G., and PHILLIS, E., The migration of solutes. Bot. Rev. 3:47–71. 1937.

EFFECTS OF NUTRIENT-SOLUTION CONCENTRATION ON THE GROWTH OF MARCHANTIA POLYMORPHA[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 552

PAUL D. VOTH

Introduction

Following forest fires, and even burns of local extent, *Marchantia polymorpha* commonly invades the bare area, forming an almost pure stand (6, and others). Dry heat is known to make available greater quantities of solutes, which may inhibit green plants such as oats but stimulate the growth of *Pyronema*, a fungus (23). Since little is known concerning the concentration of a complete nutrient solution which would be optimum for a liverwort such as *Marchantia*, the following experiments were performed.

In tests conducted as preliminary to those already reported (31, 32), it was established that a complete nutrient solution with an osmotic concentration of about 0.75–1.00 atmospheres, as used in greenhouse culture of common vegetable plants, was not conducive to maximum growth of *Marchantia*. At that time (1939) it was estimated that a dilution of approximately one-fifth was a more desirable solution. The present report is apparently the first to determine the dilution which is optimum for the growth of this species of *Marchantia*. Since the relative concentrations of the common ions had already been determined in this laboratory, a series of solutions were devised in which the relative proportions of the various salts remained constant but the amount varied according to a definite plan.

The problem of varying the concentration of a balanced or complete nutrient solution, with consequent changes in osmotic concentration, diffusion rates, and total salts available has been considered for many years. Experiments concerned with varying the amount of one or two of the constituents of a nutrient solution usually introduce several additional problems (15). Results when the amount of salts is changed proportionately must be judged by the range of concentration employed, the kind of plant, the season of the trials, the substrate used (whether sand or liquid culture), and other factors. A summary of such investigations has been made by TOTTINGHAM (29). LIVINGSTON (15), SHIVE (24), and others have reported earlier experiments dealing with the effect of varying the concentration of all salts. In general, it was found that the weaker or intermediate nutrient solutions produced plants superior in size or dry weight (1, 713.4 p.p.m.; 2, 0.3 per cent "normal" solution; 3, 300 p.p.m. in water culture, 2500 p.p.m. in sand; 9, 1.5 atmospheres of pressure; 10, 0.6 atmospheres or less; 20, 1/50 dilution of Knop's solution; 22, 2880 p.p.m.; 25, 1.75 atmospheres; and 27, salt concentration approximately equal to solution 5 of present report). Plants utilized for these experiments were tobacco, oats, wheat, tomato, barley, duckweeds, buckwheat, and pineapple, with the cereal grains predominating. On the other hand, several workers maintain that the solutions with higher salt content produced optimum growth (4, 3000 p.p.m.; 7, 8, 1142 p.p.m.; 19, 2200 p.p.m.; and 28, all utilizing water cultures). It is noted that solutions considered concentrated by some are classed as intermediate by others. A few workers favor the view that the concentrations of nutritive salts can

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vary widely before any noticeable effect is demonstrated (11, 1433–5337 p.p.m.) or that such fluctuations are relatively unimportant (13). In several of the investigations just mentioned solutions leached from the soil were employed (7, 8, 22).

While dry weights of plants or plant parts and similar data are important in the determination of the optimum solution, anatomical investigations often supplement or even change the interpretation of data. LIVINGSTON (15) noted that the usual branched filament of the alga *Stigeoclonium* was supplanted by spherical colonies of cells when strong solutions of inorganic salts were used as a growing medium. SCHOENE (21) demonstrated that no rhizoids are produced on newly-formed protonemata of *Funaria* and other mosses. The most critical studies of NIGHTINGALE and FARNHAM (16) established that dilute nutrient solutions resulted in vigorously growing, succulent sweet-pea plants. The failure of cells to mature rapidly, especially at the base of the pedicel, was responsible for the formation of an abscission layer and subsequent loss of the flower. HAYWARD and LONG (9) demonstrated that in tomato high nutrient concentrations favored rapid maturation, especially of vascular tissues, with consequent smaller cells and organs.

A correlation between gross appearance, weights, area, and anatomical changes of *Marchantia* is the object of the experiments reported here.

Material and methods

Clones of male and female plants of *Marchantia polymorpha*, designated cultures A and B, respectively (32), were again used in these experiments. The culture method described previously (31) consisted of placing six plants on a disk of glass cloth supported by a glass rack and placed in the open half of a large moist chamber. Plants and apparatus were pressure-rinsed with distilled water daily, after which they were supplied with 150 cc. of the appropriate nutrient solution. The present investigation is designated experiment 11 and was conducted in the greenhouse from March 29 to April 25–26, 1941 (27 days). A photoperiod of 17 hours was provided by supplementing daylight with 200-watt Mazda filament bulbs in reflectors (32). The bulbs were automatically lighted from 6:00 P.M. to midnight.

The basic solution was determined by means of nutrient triangle experiments and was suggested in a previous paper (32, p. 325). This solution is designated number 5 in table 1. By using higher as well as lower relative proportions of the fundamental solution (number 5), ten solutions were prepared with the extremes of concentrations ranging from 160 to 1. Solution 2 is of approximately the same concentration as nutrient solutions used to grow greenhouse plants such as beans, tomatoes, etc. Solution 1 is twice as concentrated as solution 2; and beginning with solution 6, succeeding dilutions are by one-half.

Osmotic concentrations were determined cryoscopically, using freshly prepared solutions. The hydrogen-ion concentrations were made with an instrument equipped with a glass electrode. The higher dilutions tended to be slightly more acid than less dilute ones, probably because of the acidity of the distilled water. When left standing for several weeks in covered, glazed earthenware containers, the solutions tended to become more alkaline, with proportionally larger increases as dilutions were greater.

The molar concentration of solution 5 is: K 0.0012, Ca 0.0007, Mg 0.0014, NO_3

0.0034, PO_4 0.0004, and SO_4 0.0008 mols per liter. These ten solutions did not remain stable when plants were actively growing on them. Owing to the high nitrate content and the low phosphate concentration, the hydrogen-ion concentration decreased rapidly soon after the solution was supplied to the plant. In several stock cultures, readings of pH 10.0 or greater were recorded without pronounced detrimental effects to the plants. This tendency toward alkalinity is well known (30). When a solution can be used in which KH_2PO_4 or other phosphate salts (17) are relatively high, and even greater in amount than any other salt (26), this fluctuation in pH is greatly diminished by the buffer action of the phosphate.

Investigation

Weight, area, and gemmae cups

Each *Marchantia* cutting, consisting of one apical notch and surrounding tissue, averaged 3 sq. cm. in area when planted on March 29. Each group of six plants received daily portions of 150 cc. of solution 5 for the first 3 days, since it was known that this nutrient combination promoted rhizoid formation. It has been used successfully for several years in growing the stock plants. Following this preliminary treatment, the specified variations of this solution (table 1) were supplied each culture of six plants again at a rate of 150 cc. per day.

After about the tenth day, plants supplied with solutions 1 and 2 became

TABLE 1

QUALITIES OF NUTRIENT SOLUTION EMPLOYED IN EXPERIMENT 11 (MARCH–APRIL, 1941)

Solution no.	Relative concentration of solutions		Molar concentration of salts (millimols)					Concentration of micronutrients (p.p.m.)	Osmotic concentration in atmospheres (average)	pH (average)
	Decimal	Fractional	KNO_3	$Ca(NO_3)_2 \cdot 4H_2O$	$Mg(NO_3)_2 \cdot 6H_2O$	KH_2PO_4	$MgSO_4 \cdot 7H_2O$			
1....	1.00	$\frac{160}{160}$	8.0	7.0	6.0	4.0	8.0	2.0*	1.83	4.93
2....	0.50	$\frac{80}{160}$	4.0	3.5	3.0	2.0	4.0	1.0	0.94	5.14
3....	0.30	$\frac{48}{160}$	2.4	2.1	1.8	1.2	2.4	0.6	0.59	5.38
4....	0.20	$\frac{32}{160}$	1.6	1.4	1.2	0.8	1.6	0.4	0.37	5.66
5....	0.10	$\frac{16}{160}$	0.8	0.7	0.6	0.4	0.8	0.2	0.21	5.94
6....	0.075	$\frac{12}{160}$	0.6	0.525	0.45	0.3	0.6	0.15	0.18	5.92
7....	0.050	$\frac{8}{160}$	0.4	0.350	0.30	0.2	0.4	0.10	0.13	6.05
8....	0.025	$\frac{4}{160}$	0.2	0.175	0.15	0.1	0.2	0.05	0.08	6.03
9....	0.0125	$\frac{2}{160}$	0.1	0.0875	0.075	0.05	0.1	0.025	0.05	6.09
10....	0.00625	$\frac{1}{160}$	0.05	0.04375	0.0375	0.025	0.05	0.0125	0.01	5.95

* Concentration of $MnSO_4$, $ZnCl_2$, and $Na_2B_4O_7$, singly; concentration of $FeSO_4$ is 0.1 of the figure given.

slightly translucent and were definitely smaller than plants on more dilute solutions. Plants on solutions 9 and 10 gradually developed red-purple scales, rhizoids, and ventral epidermis after a similar period. The development of translucency in plants grown on nutrient solutions of high concentration and the anthocyanin coloration of plants supplied with solutions of lesser salt content continued until the end of the experiment. Plants growing on solutions containing intermediate amounts of salts were generally the light to dark green color characteristic of *Marchantia*. The color and the general aspect of the plants at the end of the experiment may be summarized as follows:

SOLUTION 1.—Thalli were watery; wings and even tips of many plants were dead. Tips of surviving plants grew into elongated thalli, resembling regenerated tips of overwintered or injured plants commonly seen in discarded stock cultures.

SOLUTION 2.—Low-lying plants were watery, others were bright yellow-green. Tips generally were broad, and all plants were alive.

SOLUTIONS 3, 4, 5.—With decreasing salt content the plants tended to increase in size, to become darker in color, to possess ascending tips, and to develop rhizoids more abundantly.

SOLUTIONS 6, 7, 8, 9.—The plants became increasingly darker red-purple (rhizoids, scales, and lower epidermis) and decreased slightly in size with decreased concentration of the solution. Rhizoids were produced in increasing quantities, and thalli were brash.

SOLUTION 10.—The excessive development of anthocyanin on the ventral surface and the yellow-green of the dorsal surface indicated that the lack of an adequate nitrate supply was more pronounced than the lack of phosphate. The smaller number of gemmae cups and the narrow thallus with few dichotomies also expressed nitrate more than phosphate deficiency. These plants much resembled those of previous experiments in which nitrates and phosphates were lacking (31).

Table 2 shows the various trends of growth. Fresh and dry weights tend to increase progressively from solutions 1 to 6 and to decrease from solutions 7 to 10. Plants grown on solution 5 had a total fresh weight of 18×8.518 gm., which was slightly higher than the weight of plants on solution 6 (18×8.096 gm.). The greater weight of plants on solution 5 in this instance may be attributed to the very favorable growth of all female plants on the latter solution. The fresh weights of the male plants and the calculated dry weights of both sexes were all greater when plants were growing on solution 6. When actual dry weights of plants growing on the two solutions are known and compared, both sexes produced more substance when grown on solution 5. Male plants in one culture dish supplied with solution 5 grew poorly, which, at least in part, accounts for the failure of solution 5 to be optimum by all methods of calculation.

In area, the female plants on solution 5 exceeded similar plants on solution 6. In all other comparisons of area, solution 6 is optimum to solution 5. As shown in table 2, male plants on solution 4 were slightly larger on the average than similar ones on solution 5 but did not approach the average area of male plants on solution 6.

Gemmae cups vary greatly from plant to plant, but a comparison of the totals of averages based on eighteen plants (table 2) indicates that the maximum number was produced on solution 4. For both

TABLE 2

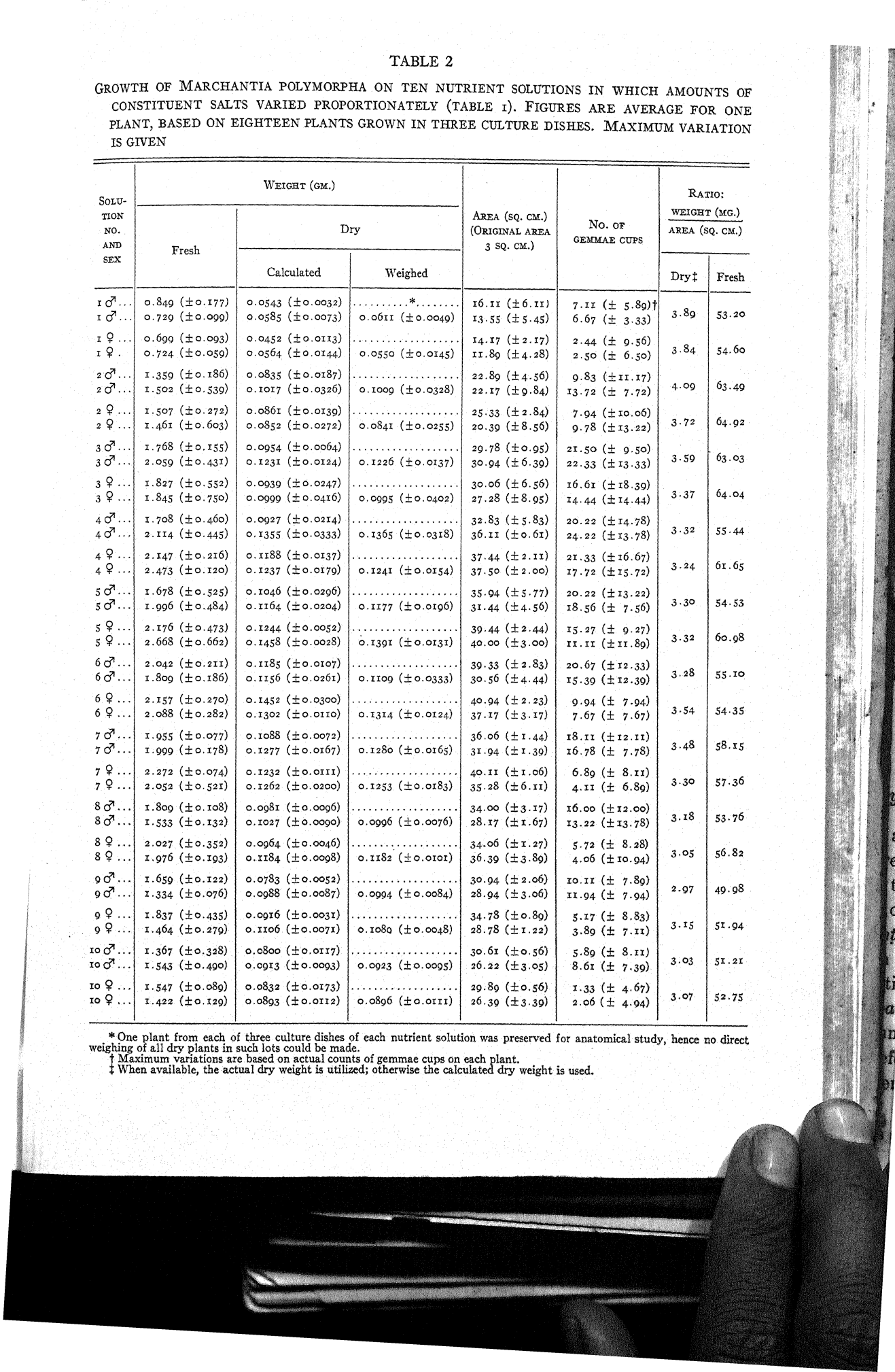
GROWTH OF MARCHANTIA POLYMORPHA ON TEN NUTRIENT SOLUTIONS IN WHICH AMOUNTS OF CONSTITUENT SALTS VARIED PROPORTIONATELY (TABLE 1). FIGURES ARE AVERAGE FOR ONE PLANT, BASED ON EIGHTEEN PLANTS GROWN IN THREE CULTURE DISHES. MAXIMUM VARIATION IS GIVEN

Solution no. and sex	Weight (gm.)			Area (sq. cm.) (Original area 3 sq. cm.)	No. of gemmae cups	Ratio: weight (mg.) / area (sq. cm.)	
	Fresh	Dry					
		Calculated	Weighed			Dry‡	Fresh
1 ♂ ...	0.849 (±0.177)	0.0543 (±0.0032)	.....*.....	16.11 (±6.11)	7.11 (± 5.89)†	3.89	53.20
1 ♂ ...	0.729 (±0.099)	0.0585 (±0.0073)	0.0611 (±0.0049)	13.55 (±5.45)	6.67 (± 3.33)		
1 ♀ ...	0.699 (±0.093)	0.0452 (±0.0113)	.....	14.17 (±2.17)	2.44 (± 9.56)	3.84	54.60
1 ♀ .	0.724 (±0.059)	0.0564 (±0.0144)	0.0550 (±0.0145)	11.89 (±4.28)	2.50 (± 6.50)		
2 ♂ ...	1.359 (±0.186)	0.0835 (±0.0187)	.....	22.89 (±4.56)	9.83 (±11.17)	4.09	63.49
2 ♂ ...	1.502 (±0.539)	0.1017 (±0.0326)	0.1009 (±0.0328)	22.17 (±9.84)	13.72 (± 7.72)		
2 ♀ ...	1.507 (±0.272)	0.0861 (±0.0139)	.....	25.33 (±2.84)	7.94 (±10.06)	3.72	64.92
2 ♀ ...	1.461 (±0.603)	0.0852 (±0.0272)	0.0841 (±0.0255)	20.39 (±8.56)	9.78 (±13.22)		
3 ♂ ...	1.768 (±0.155)	0.0954 (±0.0064)	.....	29.78 (±0.95)	21.50 (± 9.50)	3.59	63.03
3 ♂ ...	2.059 (±0.431)	0.1231 (±0.0124)	0.1226 (±0.0137)	30.94 (±6.39)	22.33 (±13.33)		
3 ♀ ...	1.827 (±0.552)	0.0939 (±0.0247)	.....	30.06 (±6.56)	16.61 (±18.39)	3.37	64.04
3 ♀ ...	1.845 (±0.750)	0.0999 (±0.0416)	0.0995 (±0.0402)	27.28 (±8.95)	14.44 (±14.44)		
4 ♂ ...	1.708 (±0.460)	0.0927 (±0.0214)	.....	32.83 (±5.83)	20.22 (±14.78)	3.32	55.44
4 ♂ ...	2.114 (±0.445)	0.1355 (±0.0333)	0.1365 (±0.0318)	36.11 (±0.61)	24.22 (±13.78)		
4 ♀ ...	2.147 (±0.216)	0.1188 (±0.0137)	.....	37.44 (±2.11)	21.33 (±16.67)	3.24	61.65
4 ♀ ...	2.473 (±0.120)	0.1237 (±0.0179)	0.1241 (±0.0154)	37.50 (±2.00)	17.72 (±15.72)		
5 ♂ ...	1.678 (±0.525)	0.1046 (±0.0296)	.....	35.94 (±5.77)	20.22 (±13.22)	3.30	54.53
5 ♂ ...	1.996 (±0.484)	0.1164 (±0.0204)	0.1177 (±0.0196)	31.44 (±4.56)	18.56 (± 7.56)		
5 ♀ ...	2.176 (±0.473)	0.1244 (±0.0052)	.....	39.44 (±2.44)	15.27 (± 9.27)	3.32	60.98
5 ♀ ...	2.668 (±0.662)	0.1458 (±0.0028)	0.1391 (±0.0131)	40.00 (±3.00)	11.11 (±11.89)		
6 ♂ ...	2.042 (±0.211)	0.1185 (±0.0107)	.....	39.33 (±2.83)	20.67 (±12.33)	3.28	55.10
6 ♂ ...	1.809 (±0.186)	0.1156 (±0.0261)	0.1109 (±0.0333)	30.56 (±4.44)	15.39 (±12.39)		
6 ♀ ...	2.157 (±0.270)	0.1452 (±0.0300)	.....	40.94 (±2.23)	9.94 (± 7.94)	3.54	54.35
6 ♀ ...	2.088 (±0.282)	0.1302 (±0.0110)	0.1314 (±0.0124)	37.17 (±3.17)	7.67 (± 7.67)		
7 ♂ ...	1.955 (±0.077)	0.1088 (±0.0072)	.....	36.06 (±1.44)	18.11 (±12.11)	3.48	58.15
7 ♂ ...	1.999 (±0.178)	0.1277 (±0.0167)	0.1280 (±0.0165)	31.94 (±1.39)	16.78 (± 7.78)		
7 ♀ ...	2.272 (±0.074)	0.1232 (±0.0111)	.....	40.11 (±1.06)	6.89 (± 8.11)	3.30	57.36
7 ♀ ...	2.052 (±0.521)	0.1262 (±0.0200)	0.1253 (±0.0183)	35.28 (±6.11)	4.11 (± 6.89)		
8 ♂ ...	1.809 (±0.108)	0.0981 (±0.0096)	.....	34.00 (±3.17)	16.00 (±12.00)	3.18	53.76
8 ♂ ...	1.533 (±0.132)	0.1027 (±0.0090)	0.0996 (±0.0076)	28.17 (±1.67)	13.22 (±13.78)		
8 ♀ ...	2.027 (±0.352)	0.0964 (±0.0046)	.....	34.06 (±1.27)	5.72 (± 8.28)	3.05	56.82
8 ♀ ...	1.976 (±0.193)	0.1184 (±0.0098)	0.1182 (±0.0101)	36.39 (±3.89)	4.06 (±10.94)		
9 ♂ ...	1.659 (±0.122)	0.0783 (±0.0052)	.....	30.94 (±2.06)	10.11 (± 7.89)	2.97	49.98
9 ♂ ...	1.334 (±0.076)	0.0988 (±0.0087)	0.0994 (±0.0084)	28.94 (±3.06)	11.94 (± 7.94)		
9 ♀ ...	1.837 (±0.435)	0.0916 (±0.0031)	.....	34.78 (±0.89)	5.17 (± 8.83)	3.15	51.94
9 ♀ ...	1.464 (±0.279)	0.1106 (±0.0071)	0.1089 (±0.0048)	28.78 (±1.22)	3.89 (± 7.11)		
10 ♂ ...	1.367 (±0.328)	0.0800 (±0.0117)	.....	30.61 (±0.56)	5.89 (± 8.11)	3.03	51.21
10 ♂ ...	1.543 (±0.490)	0.0913 (±0.0093)	0.0923 (±0.0095)	26.22 (±3.05)	8.61 (± 7.39)		
10 ♀ ...	1.547 (±0.089)	0.0832 (±0.0173)	.....	29.89 (±0.56)	1.33 (± 4.67)	3.07	52.75
10 ♀ ...	1.422 (±0.129)	0.0893 (±0.0112)	0.0896 (±0.0111)	26.39 (±3.39)	2.06 (± 4.94)		

*One plant from each of three culture dishes of each nutrient solution was preserved for anatomical study, hence no direct weighing of all dry plants in such lots could be made.

† Maximum variations are based on actual counts of gemmae cups on each plant.

‡ When available, the actual dry weight is utilized; otherwise the calculated dry weight is used.



sexes the numbers are greater for plants growing on solution 3 than on solution 5, but both treatments produced fewer gemmae cups than plants on solution 4. In all instances where totals from eighteen or more plants are compared for number of gemmae cups, the male plants of each treatment produced a greater number of these reproductive bodies than the female ones. In this experiment a total of 3060 gemmae cups was counted on female and 5420 on male plants. This ratio of 1 to 1.44 is less than the ratios of 1 to 4 and 1 to 6.6 reported for experiments 8 and 9 and experiment 10 (**31, 32**), respectively, where solutions approximated number 5 of this experiment with respect to osmotic concentration.

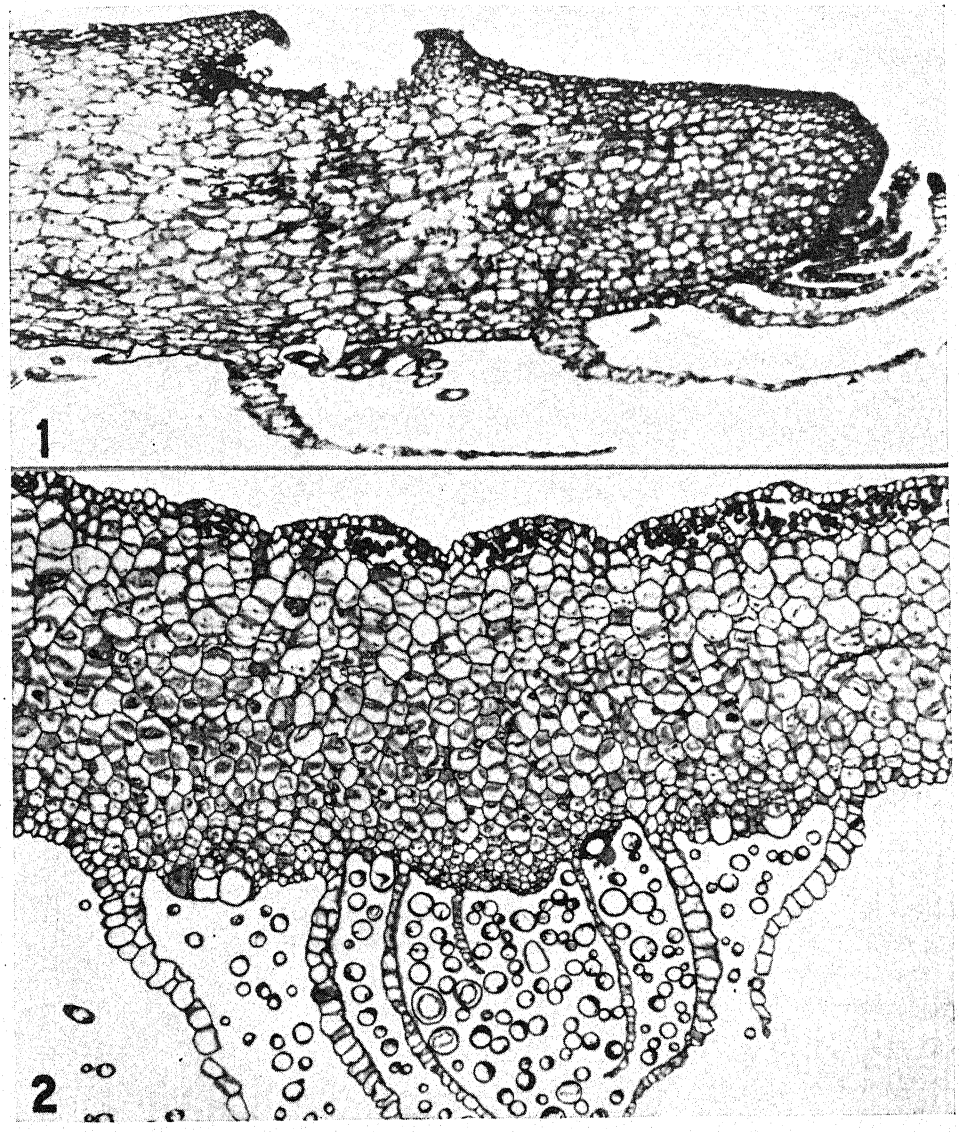
The ratios of the area to the weight, based on the averages of the plants of each sex, varied only moderately, with a tendency toward a higher ratio in plants grown on more concentrated solutions. The larger ratios (4.09 on the dry-, 64.92 on the fresh-weight basis) were found in plants of treatment 2, indicating a relatively smaller area and a relatively larger weight compared with plants growing on other treatments. Smallest ratios were secured for plants growing on treatments 8, 9, and 10.

The ability of *Marchantia* to survive great changes in hydrogen-ion concentration is evidently shared by many mosses (**12**). Suitable buffers containing no phosphate (because of increased growth on solutions low in phosphates) should be added to nutrient solutions, thus controlling a factor which has not received enough attention in the experiments reported here.

ANATOMICAL VARIATIONS

Cross sections of the thallus, approximately 2 mm. back from the apical notch, and longitudinal sections through the notch, were mounted and stained by the usual procedure. Sections were 7μ in thickness. Sixty plants were investigated in this manner. Plants on concentrated nutrient solutions possessed thin cell walls which collapsed to a certain degree when preserved in formalin-acetic acid-alcohol. Figure 1 is a longitudinal section of a male plant on solution 1, passing through a gemma cup located dorsally. The apical cell is at the extreme right, just above the youngest scales. Ventrally, rhizoids and scales are distributed normally, although cells of the latter are collapsed. A very small zone of growth surrounding the apical cell contrasts with the remainder of the thallus, which has matured rapidly—as indicated by the optically empty cells. Many internal necrotic areas characterize this section and nearly all other plants growing on solution 1. The lack of gemmae in the cup and photosynthetic filaments in the air chambers is striking.

The other extreme of thallus development is shown in figure 2, a cross section of a female plant in the midrib region which was supplied with solution 10. It is characterized by optimum development of rhizoids of the smooth and tuberculate varieties, scales, storage region, photosynthetic filaments, and pores in the upper epidermis. Most cells have contents, and cell walls are unusually sturdy. No collapse of any cell is discernible. DASSONVILLE (**5**) reported that seed plants grown in distilled water possessed a more prominent cuticle and fragile cell walls. Tissues matured and lignified rapidly and early, and secondary growth was limited. In contrast, *Marchantia* gametophytes develop thicker walls when only a very small amount of salt is present in the solution. Rapid and early maturity of tissues when plants are supplied concentrated solutions is equal-



FIGS. 1, 2.—Sections through thallus of *Marchantia* near growing tip: Fig. 1, longisection of male plant on solution 1 showing necrotic areas and lack of living gemmae. Fig. 2, cross section of female plant on solution 10 showing thickened cell walls and well-developed photosynthetic filaments.

ly true in *Marchantia*, sweet pea (16), and tomato (9).

Solutions between the extremes of 1 and 10 permitted development in a manner intermediate between that of the two plants illustrated. No necrotic areas were found in plants on solutions other than 1 and 2. As solutions became progressively more dilute, the collapse of cells lessened as cell walls increased in thickness. Gemmae production in solution 2 was much greater than in the most concentrated solution, and the number of cups reached a maximum in plants on solution 3. A survey of all microscopic sections in this experiment, as well as those of previous ones, indicates that—regardless of great variations in individual nutrient salts and variations in total salt content—*Marchantia* thalli produce a storage tissue 14–20 cells in thickness.

The extreme of cell derangement is shown in figure 3, where the optically empty cells of the older part of the thallus contrast sharply with the zones of necrotic cells nearer the apex.

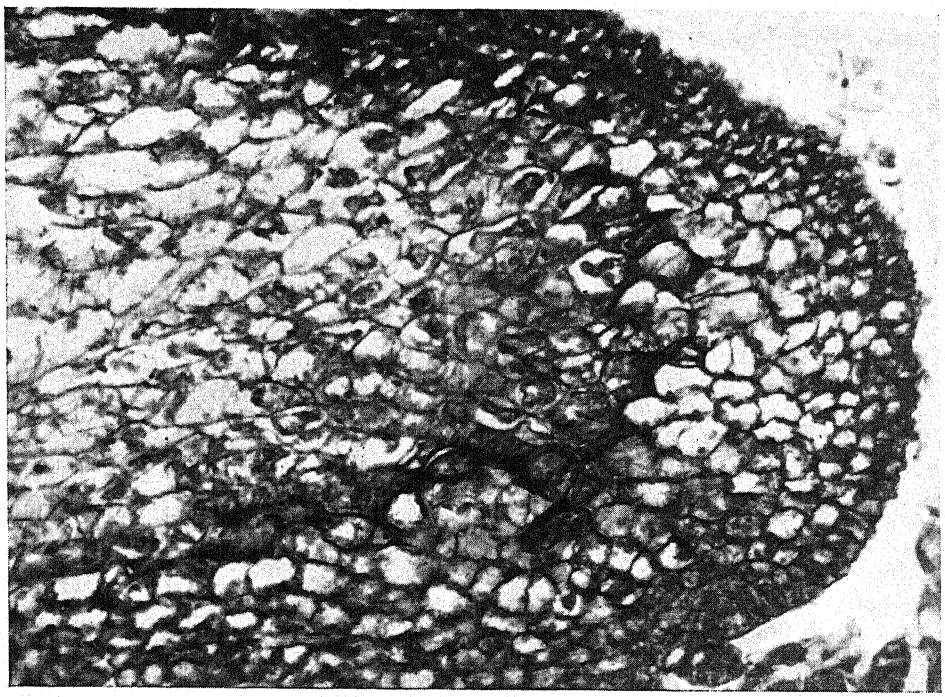


FIG. 3.—Longisection of male plant on solution 2 showing clear cells in center of thallus and concentric zone of necrotic cells. Apical cell at lower right.

Discussion

Marchantia polymorpha makes the greatest vegetative growth on solutions which have an osmotic concentration ranging from 0.21 to 0.18 atmospheres (solutions 5 and 6). That other plants grow larger on dilute solutions has been reported by SAEGER (20), who found optimum growth of duckweeds on a 1/10 dilution of Knop's solution, and by LIEBIG (14), who grew *Isoetes* from spores on diluted Knop's solution. That protonema of *Funaria* and *Ceratodon* can withstand solutions of high osmotic value is reported by PRINGSHEIM and PRINGS-

HEIM (18) and is evident from the report of SCHOENE (21). The exact conditions which *Marchantia* encounters when invading a newly-burned area has not been determined. From greenhouse experiments it is concluded that while this liverwort is hardy in extreme concentrations of nutrient solutions, its optimum vegetative development is in the dilute medium. The enormous size of some *Marchantia* plants in mountainous regions may depend on the attainment of such a dilution.

Solution 3, with an osmotic concentration of about 0.59 atmospheres, was most conducive to the development of gemmae cups (table 2). The larger number of cups on plants supplied with concentrated solutions was noted several years ago in preliminary experiments. Development of the cups evidently is dependent upon several factors when results of this and previous experiments are summarized. Four conditions contribute to greater production: (1) Relatively high osmotic concentration of the nutrient solution (table 2) results in the formation of a greater number of these reproductive bodies, especially when the smaller size of such plants is considered. (2) When phosphates are eliminated from the nutrient solutions and nitrates are abundantly supplied, a large number of cups are produced by relatively small plants (31, 32). (3) Male plants of clone A used for these experiments exceed female plants of clone B in production of the cups (table 2, and previous experiments, 31, 32). (4) More gemmae cups are produced on a short photoperiod of about 8 hours than on a long photoperiod of 17–18 hours (31).

If a common denominator exists for these factors it has not yet been determined for this plant.

Preserving one from each culture of six plants in previous experiments and from half the cultures in this one necessitated the selection of a representative plant for anatomical study and the calculation of the dry weight of six plants from the percentage dry weight of five plants and the known fresh weight of all. This method had not been checked for validity in the present series of experiments until experiment 11 permitted the culture of enough plants to make direct comparison possible. At harvest time half the cultures were treated as if one plant from each was to be preserved in an alcoholic solution. Instead it was segregated and its weight compared directly with the calculated weight (table 2). The greatest discrepancies between actual and calculated weights appeared in the averages of plants 5 ♀ and 6 ♂, with differences of 6.7 and 4.7 mg. per plant, respectively. In nearly all other cultures the dry-weight differences were only a milligram or less. About half the actual weights were higher and the other half lower than the calculated ones. With greater care in weighing fresh plants these differences should become even less.

Summary

1. Using methods previously described, cultures of two clones of *Marchantia polymorpha* were supplied with ten nutrient solutions ranging in concentration from 1.83 to 0.01 atmospheres, of which solution 5, with a concentration of 0.21 atmospheres and 0.0033 mols per liter, was optimum for vegetative growth —as judged by fresh and dry weights and area. It contained 1.6 cc. KNO_3, 1.4 cc. $Ca(NO_3)_2$, 1.2 cc. $Mg(NO_3)_2$, 0.8 cc. KH_2PO_4, and 1.6 cc. $MgSO_4$, each of a 0.5 *M* solution per liter, and the usual micro-nutrients. Solution 6 was by some standards slightly superior and by others inferior to solution 5. Its concen-

tration was 75 per cent that of solution 5. The pH characteristics of these solutions are briefly discussed.

2. Gemmae cups are most numerous on plants grown on solution 4.

3. Gemmae cups are produced on female and male plants in a ratio of 1 to 1.44—a lower ratio than in previous experiments.

4. Solutions with high salt concentration tended to kill the growing tips of plants and produced translucent thalli. Maturity was rapid, with cells slightly smaller than those grown on dilute solutions. Solutions low in salts favored the development of anthocyanin, numerous rhizoids, and a sturdy plant body possessing cells with living contents and thicker cell walls.

5. The calculation of dry weights of six plants from the weight of five and the fresh weights of six and five plants is reasonably accurate.

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LITERATURE CITED

1. AYRES, A. H., Influence of the composition and concentration of the nutrient solution on plants grown in sand cultures. Univ. Calif. Publ. Agr. Sci. 1:341–394. 1917.
2. BIRNER, (H.), and LUCANNS, (B.), Wasserculturversuche mit Hafer. Landw. Versuchsst. 8:128–177. 1866.
3. BREAZEALE, J. F., Effect of the concentration of the nutrient solution upon wheat cultures. Science n.s. 22:146–149. 1905.
4. BRENCHLEY, WINIFRED E., The effect of the concentration of the nutrient solution on the growth of barley and wheat in water cultures. Ann. Bot. 30:77–90. 1916.
5. DASSONVILLE, CH., Influence des sels minéraux sur la forme et la structure des végétaux. Rev. Gén. Bot. 10:15–25; 59–68; 102–124; 161–170; 193–199; 238–260; 289–304; 335–344; 370–380. 1898.
6. GRAFF, P. W., Invasion by *Marchantia polymorpha* following forest fires. Bull. Torr. Bot. Club 63:67–74. 1936.
7. HALL, A. D., BRENCHLEY, WINIFRED E., and UNDERWOOD, LILIAN M., The soil solution and the mineral constituents of the soil. Phil. Trans. Royal Soc. London B 204:179–200. 1914.
8. ———, ———, and ———, The soil solution and the mineral constituents of the soil. Jour. Agr. Sci. 6:278–301. 1914.
9. HAYWARD, H. E., and LONG, E. M., Anatomical and physiological responses of the tomato to varying concentrations of sodium chloride, sodium sulphate, and nutrient solutions. BOT. GAZ. 102:437–462. 1941.
10. HOAGLAND, D. R., Relation of the concentration and reaction of the nutrient medium to the growth and absorption of the plant. Jour. Agr. Res. 18:73–117. 1919.
11. ———, Optimum nutrient solutions for plants. Science n.s. 52:562–564. 1920.
12. IKENBERRY, G. J., The relation of hydrogen-ion concentration to the growth and distribution of mosses. Amer. Jour. Bot. 23:271–279. 1936.
13. KOSTYTSCHEW, S., Lehrbuch der Pflanzenphysiologie. Vol. 2. Julius Springer, Berlin. 1931.
14. LIEBIG, JOHANNA, Ergänzungen zur Entwicklungsgeschichte von *Isoetes lacustre* L. Flora 125:321–358. 1931.
15. LIVINGSTON, B. E., The role of diffusion and osmotic pressure in plants. Decennial Publ. Univ. Chicago, Ser. 2. 8:1–149. 1903.
16. NIGHTINGALE, G. T., and FARNHAM, R. B., Effects of nutrient concentration on anatomy, metabolism, and bud abscission of sweet pea. BOT. GAZ. 97:477–517. 1936.
17. PRIANISHNIKOV, D. N., and DOMONTOVITCH, M. K., The problem of a proper nutrient medium. Soil Sci. 21:327–348. 1926.
18. PRINGSHEIM, E. G., and PRINGSHEIM, OLGA, Physiologische Studien an Moosen. 3. Mitteilung. Die Züchtung von Laubmoosprotonemen im Dunkeln. Jahrb. wiss. Bot. 82:311–332. 1935.
19. REED, H. S., and HAAS, A. R. C., Some relations between the growth and composition of young orange trees and the concentration of the nutrient solution employed. Jour. Agr. Res. 28: 277–284. 1924.
20. SAEGER, ALBERT, The growth of duckweeds in mineral nutrient solutions with and without organic extracts. Jour. Gen. Physiol. 7:517–526. 1925.
21. SCHOENE, KURT, Beiträge zur Kenntnis der Keimung der Laubmoossporen und zur Biologie der Laubmoosrhizoiden. Flora 96:276–321. 1906.

22. SCOFIELD, C. S., The effect of absorption by plants on the concentration of the soil solution. Jour. Agr. Res. 35:745–756. 1927.
23. SEAVER, F. J., and CLARK, E, D., Biochemical studies on soils subjected to dry heat. Biochem. Bull. 1:413–427. 1912.
24. SHIVE, J. W., A study of physiological balance in nutrient media. Physiol. Res. 1:327–397. 1915.
25. ———, A study of physiological balance for buckwheat grown in three-salt solutions. New Jersey Agr. Exp. Sta. Bull. 319. 1917.
26. ———, The influence of sand of different degrees of fineness upon the concentration and reaction of a nutrient solution. New Jersey Agr. Exp. Sta. Report 1919:363–366. 1920.
27. SIDERIS, C. P., KRAUSS, B. H., and MASUNAGA, E., The effect of the salt concentration of the culture solution on the growth and composition of pineapple plants. Amer. Jour. Bot. 15:353–371. 1928.
28. STILES, WALTER, On the relation between the concentration of the nutrient solution and the rate of growth of plants in water culture. Ann. Bot. 29:89–96. 1915.
29. TOTTINGHAM, W. E., A quantitative chemical and physiological study of nutrient solutions for plant cultures. Physiol. Res. 1:133–245. 1914.
30. VAN ALSTINE, E., The relation of salt proportions to the growth of wheat in sand cultures. New Jersey Agr. Exp. Sta. Report 1919:366–374. 1920.
31. VOTH, P. D., and HAMNER, K. C., Responses of *Marchantia polymorpha* to nutrient supply and photoperiod. BOT. GAZ. 102:169–205. 1940.
32. VOTH, P. D., Gemmae-cup production in *Marchantia polymorpha* and its response to calcium deficiency and supply of other nutrients. BOT. GAZ. 103:310–325. 1941.

CHROMOSOME NUMBERS IN THE SUBFAMILY AURANTIOIDEAE WITH SPECIAL REFERENCE TO THE GENUS CITRUS[1]

C. A. KRUG[2]

Introduction

The orange subfamily, Aurantioideae, constitutes one of the important subdivisions of the Rutaceae, comprising, according to SWINGLE (26), who proposed a new taxonomic arrangement of this group, a total of 2 tribes, 6 subtribes, 33 genera, 201 species, and 15 varieties. It includes types which show a wide range of morphological and physiological characters, varying from xerophytic forms, as *Eremocitrus glauca* (Lindl.) Swingle, growing wild in the Australian deserts, with small, narrow, and linear leaves, to forms well adapted to humid tropical zones, as the pummelos or shaddocks (*C. grandis* Osbeck), natives of Malaysia and Polynesia; some have simple leaves with wings of varying sizes on their petioles, as *Citrus;* others have evergreen compound ones, as *Feroniella lucida* Swingle; and still others have trifoliate and deciduous ones, as *Aegle marmelos* Correa; the fruits vary from little berries, as in *Murraea exotica* L., to large hesperidia, as the well-known pummelos (*Citrus grandis* Osbeck), weighing several pounds each.

Considering the great diversity of characters in this group, considerable variation in its cytological constitution might also be expected, especially in regard to chromosome number. On the contrary, this number has been found relatively constant for all members of the subfamily so far investigated, the basic number for all of them being n = 9 and nearly all cultivated varieties being diploid (2n = 18).

[1] Paper no. 481, University of California Citrus Experiment Station, Riverside, California.

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STRASBURGER (25) was apparently the first to make cytological investigations on one of the members of this group, the genus *Citrus*, mentioning 8 as the haploid number; also OSAWA (22) gives this haploid number as the most probable for *Citrus*. All other workers, particularly LONGLEY (15, 16), FROST (3–8), and NAKAMURA (18–20), agree that 9 is the basic number. STRASBURGER'S and OSAWA'S failure to determine the correct number probably was due to defective technique. With respect to chromosome morphology, little information is available. KANDELAKI (10), investigating several species of *Citrus* and *Fortunella margarita* (Lour.) Swingle, attempted to classify the metaphase chromosomes of somatic mitosis into three groups: one with distinctly unequal-armed chromosomes, another with only slightly unequal-armed chromosomes, and a third group consisting of chromosomes with satellites. From the findings of several other investigators, including the writer, it seems unquestionable that marked size differences exist between chromosomes of the same somatic set in most of the species and genera of this subfamily so far studied.

In the present paper the chromosome numbers of several genera, species, and varieties of the Aurantioideae not previously determined are presented, and table 1 includes all determinations made so far that were found in the literature available to the writer.

Material and methods

The following determinations were made on material collected at the University of California Citrus Experiment Station, Riverside, California, in the

spring of 1941. For correct identification of this material (excluding horticultural varieties) the writer is especially indebted to Dr. W. T. SWINGLE, to whom herbarium material was submitted in Washington, D.C. Chromosome counts were made in root tips from leafy cuttings which were rooted in sand in the greenhouse; roots were fixed in Craf (23) and dehydrated by the butyl-alcohol method. Paraffin sections were stained with Heidenhain's haematoxylin. A few determinations were also made in aceto-carmine smears of young anthers.

NON-CITRUS MATERIAL.—Chromosome counts were made in the following material:

1. *Clausena lansium* (Lour.) Skeels. (C.E S. 1460, 7A–7–4; C.P.B. 2904).[3] This genus, native of South China, belongs to the tribe Clauseneae, subtribe Clauseninae; it is represented by large trees with pinnately compound leaves; its flowers are white and borne in large panicles, the fruits being small round berries. 2n = 18 (fig. 1).

2. *Atalantia citroides* Pierre ex Guill. (C.E.S. 1430, 7A–7–9; C.P.B. 7534). According to SWINGLE, the material investigated might possibly belong to *A. monophylla*, as unfortunately no flowers were available for specific identification. *Atalantia* is one of the genera of the tribe Citreae, subtribe Citrinae, being characterized by short, narrow, and thick leaves. 2n = 18 (fig. 2).

3. *Microcitrus* spp. (C.E.S. 1484, 7A–12–2; C.E.S. 1485, 7A–12–3; C.E.S. ?, 7A–14–4). Three species were examined at the Citrus Experiment Station, two of which were labeled respectively *M. australasica* var. *sanguinea* and *M. virgata*. The validity of these specific determinations could not be established. All three species have small, blunt, ovate leaves and sharp spines, characteristic of this genus. 2n = 18 (figs. 3, 4).

4. *Afraegle gabonensis* (Swingle) Engler (C.E.S. 1432, 7A–8–8; C.P.B. 7516–A). The genus belongs to the tribe Citreae, subtribe Balsamocitrinae. The Riverside specimen is a small tree with *Citrus*-like leaves, which are somewhat purplish in color; its thorns are long and stout; it does not seem to flower and fruit in California. 2n = 18.

[3] Citrus Experiment Station accession number and location (field-block-row-tree) in a Station orchard. C.P.B. and S.P.I. designate accession numbers of the U.S. Department of Agriculture.

CITRUS MATERIAL.—The following species and varieties of *Citrus*, economically the most important group, were also found to have 2n = 18:

1. *Citrus aurantifolia* Swingle: *Kusai lime[4] (C.E.S. 452, 3A–15–3); Mexican lime—considered the type species—(C.E.S. 1710, 3A–14–3) (fig. 5); Palestine Sweet lime (C.E.S. 1482, S1A–6–13; C.P.B. 1158).

2. *C. celebica* Koord. var. *southwickii* (Wester) Swingle (C.E.S. 2453, 3A–36–2; C.P.B. 10125) (fig. 6).

3. *C. hystrix* DC. (C.E.S. 2454, 3A–37–1; C.P.B. 2881).

4. *C. ichangensis* Swingle (C.E.S. 2431, S1A–10–15; C.P.B. 954) (fig. 7).

5. **C. leiocarpa* Hort. nov. var. (possibly a form of *C. tachibana* [Mak.] Tan.) (C.E.S. 2448, 7A–8–6; C.P.B. 10280) (fig. 8).

6. *C. limon* (L.) Burm.: Lisbon lemon (C.E.S. 584, S1A–4–4); *Meyer lemon (C.E.S. 601, S1A–5–15; S.P.I. 23028).

7. *C. macroptera* Montr. (C.E.S. 432, 3A–38–1) (fig. 9).

8. *C. medica* L.: Philippine citron (C.E.S. 757, 3A–8–5) (fig. 10); Italian citron (C.E.S. 128, 3B–2–5; S.P.I. 701); Indian citron (C.E.S. 138B, 3A–10–2).

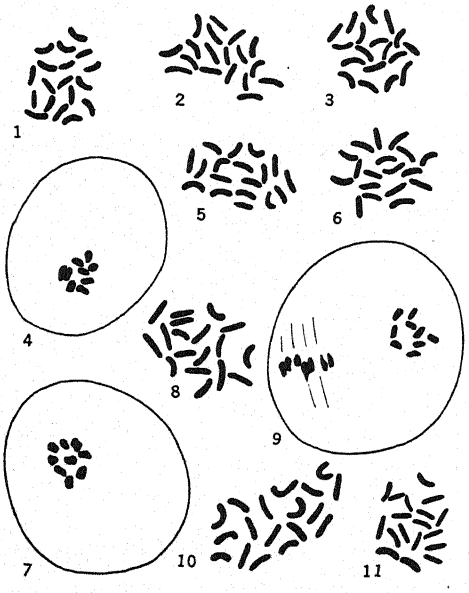
9. **C. webberi* Wester (C.E.S. 1455, 7A–9–4; C.P.B. 10026) (fig. 11).

In spite of considerable differences in morphological characters and the fact that these genera and species belong to widely separated groups in the Aurantioideae, the same chromosome number (2n = 18) was found in all of them.

In table 1 the general sequence of the new classification by SWINGLE (26) has been followed, although many specific

[4] Throughout this paper (including the table) specific and subspecific names not considered valid by SWINGLE (28) are marked with an asterisk (*) *preceding* the word and so also are names of horticultural varieties not here correctly placed by species. For further explanation, see Addendum, following the Summary.

names are not acceptable to him (personal information); for instance, many of TANAKA'S species, not recognized as such by SWINGLE, are listed as they are given by NAKAMURA (18–20). In some instances horticultural variety names are also mentioned, as these may be useful to investigators of *Citrus*. To make the list as complete as possible, the writer's new determinations have also been included. In this table, tetraploid forms known to be nucellar-seedling derivatives of particular horticultural varieties have been named (by H. B. FROST) by prefixing "Tetraploid" as a part of the varietal name.



FIGS. 1–11.—Fig. 1, *Clausena lansium* (Lour). Skeels; metaphase chromosomes. 2n = 18. Fig. 2, *Atalantia citroides* Pierre ex Guill.; metaphase chromosomes. 2n = 18. Fig. 3, *Microcitrus* sp. (possibly *australasica* var. *sanguinea*); metaphase chromosomes. 2n = 18. Fig. 4, *Microcitrus* sp.; first metaphase of meiosis. n = 9. Fig. 5, *Citrus aurantifolia* Swingle (Mexican lime); metaphase chromosomes. 2n = 18. Fig. 6, *C. celebica* Koord. var. *southwickii* (Wester) Swingle; metaphase chromosomes. 2n = 18. Fig. 7, *C. ichangensis* Swingle; first metaphase of meiosis. n = 9. Fig. 8, **C. leiocarpa* Hort. var. nov. (*C. tachibana?*); metaphase chromosomes. 2n = 18. Fig. 9, *C. macroptera* Montr.; second metaphase of meiosis. n = 9. Fig. 10, *C. medica* L. (Philippine citron); metaphase chromosomes. 2n = 18. Fig. 11, **C. webberi* Wester; metaphase chromosomes. 2n = 18.

Of the thirty-three genera in this subfamily, the chromosomal constitution of only eleven is known; all these have 9 as their basic number. With regard to the occurrence of polyploidy, the following summary is presented:

TRIPLOIDS.—More than 50 triploids, or approximate triploids, have been found in the genus *Citrus*. Several triploids were hybrids between a diploid *Citrus-Fortunella* hybrid and tetraploid *Fortunella* (13, 25); about forty were hybrids between diploid *Citrus* varieties, their origin being assumed to be due to duplication of chromosomes, mainly in the nucellus, before the formation of the egg cell, perhaps through nonreduction in megasporocytes (5); twelve (LAPIN) were seedlings from open pollination; and only two, the Tahiti and Bearss limes (*C. aurantifolia* Swingle) have been recently found to be triploid varieties of economic interest. Triploids have been produced by seed parents of five *Citrus* species (if *C. paradisi* is ranked as a species).

TETRAPLOIDS.—These have so far been found in four genera (*Triphasia*, *Fortunella*, *Poncirus*, and *Citrus*); they have been found more frequently in *Citrus* (appearing in at least four species [five with *C. paradisi*], according to SWINGLE'S classification, and in numerous horticultural varieties), probably because this genus is larger and has been more extensively investigated than the others.

HIGHER POLYPLOIDS.—Beyond tetraploidy, apparently only two other forms have been detected: a hybrid pentaploid orange (BACCHI, unpublished) and a hexaploid lemon, the latter from unguarded pollination of the Ponderosa lemon (14).

TABLE 1

CHROMOSOME NUMBERS IN THE AURANTIOIDEAE

MATERIAL	No. of chromosomes		AUTHOR AND YEAR
	Gametic	Somatic	
Tribe 1: Clauseneae			
Subtribe 2: Clauseninae			
Clausena lansium (Lour.) Skeels	.....	18	Krug 1943
Murraya paniculata (L.) Jack	9	.....	Toxopeus(31) 1933
Tribe 2: Citreae			
Subtribe 1: Triphasiinae			
Triphasia trifolia (Burm.) P. Wilson	9	.....	Longley (15) 1925
(Tetraploid form)	18	.....	Longley, cited in Traub and Robinson (32) 1937?
Subtribe 2: Citrinae			
Severinia buxifolia (Poir.) Tenore	9	.....	Longley (15) 1925
Citropsis schweinfurthii (Engl.) Swing. & M. K.	9	.....	Longley (15) 1925
Atalantia citroides Pierre ex Guill. (possibly *A. monophylla* DC.)	.....	18	Krug 1943
**Fortunella crassifolia* Swingle	9	.....	Longley (15) 1925
F. hindsii Swingle (4n)	18	.....	Longley (15) 1925
F. hindsii (2n)	9	.....	Longley (27) 1929
F. japonica Swingle	9	.....	Longley (15) 1925
F. margarita (Lour.) Swingle	9	.....	Longley (15) 1925
Poncirus trifoliata (L.) Raf.	9	.....	Longley (15) 1925
(Tetraploid)	.....	36	Lapin (14) 1937
Microcitrus australis (Planch.) Swing.	9	.....	Longley, cited in Traub and Robinson (32) 1937?
Microcitrus sp.	.....	18	Krug 1943
M. virgata (?)*	.....	18	Krug 1943
M. australasica var. *sanguinea* (?)	.....	18	Krug 1943
Citrus aurantifolia Swingle, lime (three varieties)	9	.....	Longley (15) 1925
Lime	9	.....	Nakamura (20) 1934
Limão Seda	.....	18	Bacchi (1) 1940
Rio Claro	.....	18	Bacchi (1) 1940
*Rangpur lime, Cravo or Rosa	.....	18	Bacchi (1) 1940
*Kusai lime	.....	18	Krug 1943
Mexican lime	.....	18	Krug 1943
Palestine Sweet lime	.....	18	Krug 1943
(3n) Tahiti lime	.....	27	Bacchi (1) 1940
Progeny of Tahiti lime	.....	19	Krug and Bacchi (11) (In press)
	.....	20	Krug and Bacchi (11) (In press)
	.....	21	Krug and Bacchi (11) (In press)
(3n) Bearss lime	.....	27	Krug and Bacchi (11) (In press)
C. aurantium L., sour orange	8	.....	Strasburger (25) 1907
(Four varieties)	9	.....	Nakamura (20) 1934
Sour orange or Agro-Sevilhana	.....	18	Bacchi (1) 1940
C. aurantium, *subspecies *bergamia* (Risso & Poiteau) (Wright & Arn. ?)	9	.....	Nakamura (20) 1934
C. celebica Koord. var. *southwickii* (Wester) Swingle	.....	18	Krug 1943
**C. deliciosa* Ten. (tetraploid)	.....	36	Lapin (14) 1937
**C. depressa* Hayata	9	.....	Nakamura (20) 1934
**C. erythrosa* Hort. ex Tanaka	9	.....	Nakamura (20) 1934
**C. genshokan* Hort. ex Tanaka	9	.....	Nakamura (20) 1934
**C. glaberima* Hort. ex Tanaka	9	.....	Nakamura (20) 1934

* *M. virgata* is a name never regularly published but tentatively assigned to a form later determined to be *M. australis*×*M. australasica* (28).

TABLE 1—*Continued*

Material	No. of chromosomes		Author and year
	Gametic	Somatic	
Tribe 2: Citreae—*Continued*			
C. grandis Osbeck, Frizzelle pummelo†	9	.....	Frost (unpublished) (1927)
(4n) Tetraploid Frizzelle pummelo	18	.....	Frost (7) 1927
Djeroek bali	9	.....	Toxopeus (31) 1933
(Seven Oriental varieties of pummelo)	9	.....	Nakamura (20) 1934
C. hystrix DC.	9	.....	Krug 1943
C. ichangensis Swingle	9	.....	Krug 1943
**C. junos* Sieb. sec. Tanaka, Yuzu and four other varieties	9	.....	Nakamura (20) 1934
**C. kinokuni* Hort. ex Tanaka	9	.....	Nakamura (20) 1934
**C. kotokan* Hayata (two varieties)	9	.....	Nakamura (20) 1934
**C. leiocarpa* Hort. (two varieties)	9	.....	Nakamura (20) 1934
Nov. var. (possibly a form of *C. tachibana* [Mak.] Tan.)	.....	18	Krug 1943
**C. limetta* Risso, Sweet lemon	9	.....	Nakamura (20) 1934
Lemon-shaped lumia	9	.....	Nakamura (20) 1934
C. limon (L.) Burm., Eureka lemon	9	.....	Longley (15) 1925
Genoa lemon	9	.....	Nakamura (20) 1934
Sicily lemon	9	.....	Nakamura (20) 1934
(Several other varieties of lemon)	9	.....	Nakamura (20) 1934
Rough lemon (see *C. verucosa*)	.....	18	Bacchi (1) 1940
Limão Doce	.....	18	Bacchi (1) 1940
Lisbon lemon	.....	18	Krug 1943
Limone Interdonato	9	.....	Ruggieri (24) 1935
Lisbon lemon	9	.....	Frost (6) 1926
(4n) Tetraploid Lisbon lemon	18	.....	Frost (4) 1925
Seedlings of Ponderosa lemon (see *C. piriformis*)	.....	18	Lapin (14) 1937
(4n, 6n) Seedlings of Ponderosa lemon	.....	36	Lapin (14) 1937
	.....	54	Lapin (14) 1937
(3n) (Four seedlings from open pollination)‡	.....	27	Lapin (14) 1937
Variegated Pink Eureka lemon§	9	.....	Frost (unpublished) (1942)
**C. limonia* Osbeck, Kusaie lime	9	.....	Nakamura (20) 1934
Rangpur lime	9	.....	Nakamura (20) 1934
Japansche citroen	9	.....	Toxopeus (31) 1933
var. *otahitensis* Tanaka, Otaheite orange	9	.....	Nakamura (20) 1934
var. *khatta* Tanaka, Khatta orange	9	.....	Nakamura (20) 1934
C. macroptera Montr.	9	.....	Krug 1943
C. medica L., citron	8	.....	Strasburger (25) 1907
	9	.....	Longley (15) 1925
var. *sarcodactylis* Swingle	9	.....	Nakamura (20) 1934
Cidra	.....	18	Bacchi (1) 1940
Philippine citron	.....	18	Krug 1943
Italian citron	.....	18	Krug 1943
Indian citron	.....	18	Krug 1943
Chinese lemon‖	9	.....	Frost (unpublished) (1942)
**C. medioglobosa* Tan.	9	.....	Nakamura (20) 1934
**C. microcarpa* Bunge, calamondin (two varieties)	9	.....	Nakamura (20) 1934
(Tetraploid calamondin)	18	.....	Nakamura (19) 1934
**C. mitis* Blanco, calamondin	9	.....	Longley (15) 1925
	.....	18	Bacchi (1) 1940
**C. natsudaidai* Hayata	9	.....	Nakamura (20) 1934

† The Frizzelle is a noncommercial polyembryonic variety with characters of both *Citrus grandis* and *C. paradisi*. See Addendum.
‡ Lapin (14) also obtained five triploid seedlings from open pollination of Shiva-mikan (species?).
§ Of unknown origin, but apparently identical, except in fruit color, with a bud-variation strain derived from the Eureka.
‖ A variety (C.E.S. 710) which seems predominantly citron in its characteristics, although the fruit is strikingly lemon-like.

TABLE 1—*Continued*

Material	No. of chromosomes		Author and year
	Gametic	Somatic	
Tribe 2: Citreae—*Continued*			
**C. nobilis* Lour. King mandarin (or orange)	9	.....	Frost (unpublished) (1927)
King mandarin	9	.....	Nakamura (20) 1934
(4n) Tetraploid King mandarin	18	.....	Frost (6) 1926
Kunembo	9	.....	Nakamura (20) 1934
**C. obovata* Rafin.	9	.....	Nakamura (20) 1934
**C. oleocarpa* Hort. ex Tanaka	9	.....	Nakamura (20) 1934
**C. paradisi* Macf. Duncan grapefruit	9	.....	Longley (15) 1925
Marsh grapefruit	9	.....	Longley (15) 1925
Marsh grapefruit (Seedy strain) (?)¶	9	.....	Frost (unpublished) (1926)
(4n) Tetraploid Marsh Seedy (?) grapefruit	18	.....	Frost (6) 1926
Imperial grapefruit	9	.....	Frost (3) 1925
(4n) Tetraploid Imperial grapefruit	18	.....	Frost (7) 1927
(4n) Tetraploid Hall grapefruit	18	.....	Frost (unpublished) (1938)
(4n) Tetraploid McCarty grapefruit	.....	36	Bacchi (unpublished)
(3n) (Seedling from open pollination)	.....	27	Lapin (14) 1937
**C. piriformis* Hassk., Ponderosa lemon (a hybrid ?)	9	.....	Nakamura (20) 1934
**C. poonensis* Hort. ex Tanaka, Ponkan	9	.....	Nakamura (20) 1934
C. reticulata Blanco, Dancy tangerine	9	.....	Longley (15) 1925
(4n) Tetraploid Dancy tangerine	18	.....	Frost (7) 1927
Willow Leaf mandarin	9	.....	Longley (15) 1925
(4n) Tetraploid Willow Leaf mandarin	18	.....	Frost (7) 1927
Unshû (=satsuma)	8	.....	Osawa (22) 1912
Satsuma orange	9	.....	Longley (15) 1925
Owari satsuma	9	.....	Nakamura (18) 1929
Wase satsuma	9	.....	Nakamura (18) 1929
Owari (?) satsuma	9	.....	Frost (unpublished) (1927)
(4n) Tetraploid Owari (?) satsuma	18	.....	Frost (7) 1927
(4n) Tetraploid satsuma	.....	36	Lapin (14) 1937
C. sinensis Osbeck, sweet orange	8	.....	Strasburger (25) 1907
Pineapple orange	9	.....	Longley (15) 1925
Valencia orange	9	.....	Longley (15) 1925
Maltese Oval orange**	9	.....	Frost (3) 1925
Paper Rind (St. Michael) orange	9	.....	Frost (3) 1925
(4n) Tetraploid Paper Rind orange	18	.....	Frost (4) 1925
Djeroek manis	9	.....	Toxopeus (31) 1933
Kinkunembo	9	.....	Nakamura (20) 1934
Lue Gim Gong orange	9	.....	Nakamura (20) 1934
Maltese Blood orange	9	.....	Nakamura (20) 1934
Parson Brown orange	9	.....	Nakamura (20) 1934
Navelencia orange	9	.....	Nakamura (20) 1934
Washington Navel orange	9	.....	Nakamura (20) 1934
(Six other varieties of orange)	9	.....	Nakamura (20) 1934
(3n) (Two seedlings from open pollination)	.....	27	Lapin (14) 1937
Laranja Doce	.....	18	Bacchi (1) 1940
Baia orange	.....	18	Bacchi (1) 1940
Imperial orange	.....	18	Bacchi (1) 1940
Ruby orange	9	.....	Frost (unpublished) (1927)
(4n) Tetraploid Ruby orange	18	.....	Frost (7) 1927
(4n) (Tetraploid orange)	.....	36	Lapin (14) 1937
Shamouti (Jaffa) orange	9	.....	Oppenheim and Frankel (21) 1929

¶ So listed, but bud-variation origin not established. For taxonomic status of grapefruit, see Addendum.

** According to Hume. This variety was formerly grown in California as Mediterranean Sweet; tree compact, semidwarf.

TABLE 1—*Continued*

Material	No. of chromosomes		Author and year
	Gametic	Somatic	
Tribe 2: Citreae—*Continued*			
**C. succosa* Hort. ex Tanaka	9	.....	Nakamura (20) 1934
**C. sunki* Hort. ex Tanaka	9	.....	Nakamura (20) 1934
**C. surcata* Hort. ex Tanaka (?)	9	.....	Nakamura (20) 1934
C. tachibana (Nak.) Tan	9	.....	Nakamura (20) 1934
**C. tamurana* Tan	9	.....	Nakamura (20) 1934
**C. tangerina* Hort. ex Tanaka (two varieties)	9	.....	Nakamura (20) 1934
**C. tankan* Hayata	9	.....	Nakamura (20) 1934
**C. verucosa* Hort. ex Tanaka (?), Rough lemon	9	.....	Nakamura (20) 1934
**C. webberi* Wester	.....	18	Krug 1943
**C. yatsushiro* Hort. ex Tanaka	9	.....	Nakamura (20) 1934
Subtribe 3: Balsamocitrinae			
Afraegle gabonensis (Swingle) Engler	.....	18	Krug 1943
Aeglopsis chevalieri Swingle	9	.....	Longley (15) 1925
Feronia limonia (L.) Swing.	9	.....	Toxopeus (31) 1933
Hybrids			
1. Sampson tangelo (*C. paradisi*×*C. reticulata*)	9	.....	Longley (15) 1925
2. Eustis limequat (*C. aurantifolia*×*Fortunella japonica*)	9	.....	Longley (15) 1925
3. Thomasville citrangequat (*Fortunella margarita*× [Willits citrange=*Poncirus trifoliata*×*C. sinensis*])	9	.....	Nakamura (20) 1934
4. Sacaton citraldin (C.E.S. 2619)	.....	18	Krug (n.d.)
5. (*Citrus aurantifolia*×*Fortunella japonica*)††×*Fortunella hindsii* (two hybrids)	$\frac{2}{2}^{7}$	.....	Longley (16) 1926; Traub and Robinson 32, p. 794) 1937
6. About twenty different hybrids of *Citrus*, from four seed-parent varieties of three species	$\frac{2}{2}^{7}$ (approximate)	.....	Frost (7, 8, and unpublished) 1927, 1930
7. *Citrus limon*×*C. grandis*	.....	27	Lapin (14) 1937
8. *Citrus limon*×*C. paradisi* (four hybrids)	.....	27	Lapin (14) 1937
9. Novoafon lemon×Meyer lemon	.....	28	Luss (17) 1935
10. *Citrus limon*×Meyer lemon (same as last?)	.....	28	Lapin (14) 1937
11. Fifteen other hybrids, from three seed-parent species	.....	27	Lapin (14) 1937
12. *Citrus sinensis* (Baianinha)×*C. sinensis* (Sabara)	.....	45	Bacchi (unpublished)
13. Clementine mandarin	9	.....	Lacarelle and Miedzyrzecki (12) 1937

†† Longley (16) gave *F. margarita* as the kumquat parent of this limequat, but Swingle (28) states that the limequat crossed with *F. hindsii* was the Eustis, which had the parentage here given. (See also Swingle, W. T., and Robinson, T. R., Jour. Agr. Res. 23:229–238. 1923.)

Aneuploids.—Only recently several cases of aneuploidy have been found; two (one?) hybrids were reported (14, 17) with 28 somatic chromosomes, having as male parent the diploid Meyer lemon.

Bacchi (1) found 28 chromosomes in another *Citrus* form, which has been omitted from table 1 because question has been raised as to its varietal and specific identification. In the progeny of this form, from open-pollinated flowers, eight seedlings have been found by Krug and Bacchi (unpublished) to have, respectively, 21, 21, 23, 29, 30, 33, 37, and 37 chromosomes. In spite of their odd numbers of chromosomes, some of these progeny plants seem to grow normally. In the progeny of the triploid Tahiti lime also, three aneuploids have been found by the same investigators, with 19, 20, and 21 chromosomes, respectively.

To the breeder of the genus *Citrus*,

valuable cytological data are therefore available, namely, the chromosome numbers of many of its species and also of most of its closest relatives. The fact that the basic number is the same for all of them has made it possible to make wide intergeneric crosses, as between *Citrus*, *Fortunella*, and *Poncirus;* the similarity of the chromosome complements of these three genera seems rather striking, as normal pairing has been reported to occur in such a trigeneric cross (**20**). This fact seems a challenge to the taxonomist, indicating that these three genera might perhaps rather be considered mere species of a single genus. Nothing is known regarding the evolutionary development in this subfamily. On the basis of the similarity of the genomes of *Fortunella*, *Citrus*, and *Poncirus*, NAKAMURA (**20**) points out that the evolution of species within the genera may be due mainly to the formation of new alleles. Further cytological investigations, supplemented by genetic analysis—the latter process being handicapped by the phenomenon of polyembryony—will doubtless help to elucidate the relationships in this important group of plants.

Summary

1. The chromosome number of fourteen species (twenty forms) belonging to five different genera of the subfamily Aurantioideae are reported. All determinations available in the literature on chromosome numbers made in this subfamily are tabulated. The basic chromosome number is n = 9 for all forms so far investigated. About 40 triploids, some 190 tetraploids (including 19 in *Poncirus*, 1 in *Triphasia*, and 1 wild form in *Fortunella*),[5] 1 pentaploid, 1 hexaploid, and 14 or 15 aneuploids[6] have been reported.

2. All these polyploids, except the 21 tetraploids just mentioned and several triploid hybrids between *Citrus* and *Fortunella*, belong to the genus *Citrus*. Considering the genetic similarity of the chromosome complements of several genera of the subtribe Citrinae, it is suggested that some of these might perhaps better be considered mere species of a single genus.

The writer expresses his indebtedness to the John Simon Guggenheim Memorial Foundation, which enabled him to spend several months at the University of California Citrus Experiment Station, Riverside, California, and to the director and staff of the divisions of Plant Breeding and Orchard Management of that Station for the many facilities provided.

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Addendum by Howard B. Frost
February, 1943

So far as *Citrus* forms which have been cytologically studied are adequately identified in publication by vernacular varietal names, they have usually been placed in this paper under the proper species as recognized by SWINGLE (**28**). It is impracticable, however, to rearrange in this way all the chromosome determinations that have been published under invalid specific names, partly because of

[5] The triploids doubtless were all gametic seedlings and therefore all genetically distinct, although in some cases several triploid seedlings had the same parentage. The tetraploids presumably were all of nucellar-seedling (apomictic) origin, except for one case of bud variation to tetraploidy (**9**). The number of distinct tetraploid forms (derived from different diploid forms) observed probably is considerably more than 20.

[6] This number includes a fragment trisomic (2n = 18 + 1 fragment) incidentally mentioned by BROWN (**2**); this is a citrange (*Poncirus trifoliata* × *Citrus sinensis*), variety not mentioned.

inadequacy of identification and partly because some forms evidently are interspecific or intergeneric hybrids. Further, some of these names supply a necessary group or varietal identification, such as is elsewhere furnished by vernacular names.

Some revision of this paper has recently been made at the University of California Citrus Experiment Station; and because of war conditions, MR. KRUG may not have opportunity to review the changes before publication. This revision is mainly based on SWINGLE's (28) monographic treatment of the Aurantioideae, which has recently become available in printer's proof. I have also added a few *Citrus* forms, partly from previously unpublished determinations.

The names marked as invalid mainly designate groups and horticultural varieties, in some cases presumptive hybrids between *Citrus* species or even involving other genera, which do not seem to deserve specific rank. Some cases deserve mention here, as follows: *C. nobilis* Lour. was based on the King mandarin or a very similar form (28); the King, however, is considered by SWINGLE to be an interspecific hybrid, perhaps between *C. reticulata* and *C. sinensis*, and therefore the mandarin species becomes *C. reticulata* Blanco (the oldest valid name). In the proof of SWINGLE's monograph, the grapefruit is distinguished as a "satellite species"; *C. paradisi*, which is closely related to the pummelo or shaddock, and the grapefruit varieties are therefore listed separately. The Rangpur and Kusaie limes are probably sour mandarins, *C. reticulata* var. *austera*, or possibly hybrids between *C. reticulata* and *C. aurantifolia*. The Meyer lemon, the Otaheite orange, the Japansche citroen (31), the yuzu (*C. junos*), and *C. webberi* all seem to be interspecific hybrids. The calamondin (*C. mitis* or *C. microcarpa*) is considered a *Citrus*×*Fortunella* hybrid. TANAKA's (30) grouping and descriptions indicate that all the following "species" are forms of *C. reticulata: deliciosa, depressa, erythrosa, genshokan, glaberima, kinokuni, poonensis, succosa*, and *tangerina;* and also that *tankan* and *natsudaidai* are closely related to *sinensis*.

In addition to the data in table 1, NAKAMURA (20) found 9 chromosomes as the gametic number in nine *Citrus* forms which he did not assign to species. LAPIN (14) reported tetraploid seedlings, not listed in table 1, from seed parents as follows: *C. grandis, C. limon, C. natsudaidai*, and *C. paradisi;* all seed parents which gave tetraploids produced mainly diploid seedlings. In LAPIN's cultures, as also in FROST's, part of the tetraploids occurred among the known nucellar (apomictically produced) seedlings accompanying hybrids from interspecific crosses, and all the rest may have been nucellar; none were found among the hybrids.

LITERATURE CITED

1. BACCHI, O., Observações citológicas em *Citrus*. I. Número de cromôsomios de algumas espécies e variedades. Jornal de Agronomia, Piracicaba 3:249–258. 1940.
2. BROWN, W. L., A modified root-tip smear technic. Stain Technol. 12:137–138. 1937.
3. FROST, H. B., The chromosomes of *Citrus*. Jour. Wash. Acad. Sci. 15:1–3. 1925.
4. ———, Tetraploidy in *Citrus*. Proc. Nat. Acad. Sci. 11:535–537. 1925.
5. ———, Polyembryony, heterozygosis and chimeras in *Citrus*. Hilgardia 1:365–402. 1926.
6, 7, 8. ———, California Agr. Exp. Sta. Ann. Rept.; Plant Breeding. 1926, 1927, 1930.
9. FROST, H. B., and KRUG, C. A., Diploid-tetraploid periclinal chimeras as bud variants in *Citrus*. Genetics 27:619–634. 1942.
10. KANDELAKI, G. V., Karyological investigations of some cultivated species and varieties of the genus *Citrus*. Trav. Inst. Bot. Tbilissi 4:109–

120. 1938. (Cit. Plant Breed. Abstr. 9:465. 1939.)
11. KRUG, C. A., and BACCHI, O., Cytological observations in *Citrus*. II. Triploid and hypertriploid varieties. In press.
12. LACARELLE, A., and MIEDZYRZECKI, CH., Nouvelle contribution à l'étude du Clementinier au Maroc. Experimentation Fruitière et Maraîchère, Rabat, Morocco. Edition Terre Marocaine. 1937.
13. LAPIN, W. K., Concerning the genotypic homogeneous stock for the *Citrus* trees. Terre Merocaine. 2:24–27. 1937. (Cit. Plant Breed. Abstr. 8:73. 1938.)
14. ———, Investigations on polyploidy in *Citrus*. U.S.S.R. All-Union Sci. Res. Institute. Humid Subtropics Works 1:1–68. 1937. (Cit. Plant Breed. Abstr. 9:339. 1939.)
15. LONGLEY, A. E., Polycary, polyspory and polyploidy in *Citrus* and *Citrus* relatives. Jour. Wash. Acad. Sci. 15:347–351. 1925.
16. ———, Triploid *Citrus*. Jour. Wash. Acad. Sci. 16:543–545. 1926.
17. LUSS, A. I., Citrus introduction and selection in the U.S.S.R. Soviet Subtropics 11:17–27. 1935. (Cit. Plant Breed. Abstr. 6:1372. 1936.)
18. NAKAMURA, MIYAWO, Cytological studies in the genus *Citrus*. I. On the Wase satsuma originated through bud variation. Studia Citrologica 3: 1–14. 1929.
19. ———, A preliminary report on the chromosome number, pollen sterility and the formation of abnormal pollen tetrads in *Citrus*. Bull. Kagoshima Coll. Agr. For. 1:11–14. 1934. (Cit. Plant Breed. Abstr. 7:234. 1937.)
20. ———, Cytological studies in the genus *Citrus*. II. The chromosome number, pollen sterility and the formation of abnormal tetrads. Studia Citrologica 6:162–178. 1934. (Cit. Plant Breed. Abstr. 8:489. 1938.)
21. OPPENHEIM, J. D., and FRANKEL, O. H., Investigations into the fertilization of the Jaffa orange. Genetica 11:369–374. 1929.
22. OSAWA, J., Cytological and experimental studies in *Citrus*. Jour. Coll. Agr. Imp. Univ. Tokyo 4:83–116. 1912.
23. RANDOLPH, L. F., A new fixing fluid and a revised schedule for the paraffin method in plant cytology. Stain Technol. 10:95–96. 1935.
24. RUGGIERI, GAETANO, Sopra i presunti rapporti genetici col limone o col cedro di una particolare varietà di limone assai resistente alla *Deuterophoma tracheiphila* Petri. R. Staz. Patolog. Veg. Roma Bol. n.s. 15:496–499. 1935.
25. STRASBURGER, EDUARD, Über die Individualität der Chromosomen und die Propfhybriden-Frage. Jahrb. wiss. Bot. 44:482–555. 1907.
26. SWINGLE, W. T., A new taxonomic arrangement of the orange subfamily, Aurantioideae. Jour. Wash. Acad. Sci. 28:530–533. 1938.
27. SWINGLE, W. T., "Kindzu" or "Golden Bean" orange (*Fortunella Hindsii*) from historic, taxonomic, and cytologic standpoints. Proc. 3d Pan-Pacific Sci. Cong. (Tokyo) 2:2001. 1929.
28. SWINGLE, W. T., The botany of *Citrus* and its wild relatives. *In* WEBBER, H. J., *et al.*, The Citrus Industry. Univ. Calif. Press, Berkeley. In press.
29. TANAKA, T., Taxonomie de la citriculture tropicale. Rev. Bot. Appl. 10:114–119. 1930.
30. TANAKA, TYÔZABURÔ, Taxonomy of the *Citrus* fruits of the Pacific region. Tanaka Citrus Exp. Sta. Mem. 1:15–36. 1927.
31. TOXOPEUS, H. J., Some cases of bud variation in *Citrus* observed on Java. Genetica 15:241–252. 1933.
32. TRAUB, H. P., and ROBINSON, T. R., Improvement of subtropical fruit crops: *Citrus*. U.S. Dept. Agr. Yearbook 1937:749–826. 1937.

INFLUENCE OF TEMPERATURE ON PHOTOPERIODIC REACTIONS IN LEAF BLADES OF BILOXI SOYBEAN

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Introduction

The writers have studied the influence on floral initiation of various combinations of night and day temperatures applied to Biloxi soybeans for a period of 5 days during which the plants received short photoperiods (5). The temperature treatments were applied to the entire plant, and it was found that a night temperature of 55° F. initiated significantly fewer flower buds following the treatment with short photoperiod than those held at night temperatures of 65°, 75°, and 85° F.[3] Significant differences between lots held at a day temperature of 55° and lots held at certain higher day temperatures were also observed, but these differences were much less than those found between lots held at high and low night temperatures. The results indicated that the interaction between photoperiod and temperature was to be explained as an influence on some process or series of processes occurring during the dark period.

The photoperiodic reaction that results in flowering in Biloxi soybean plants occurs in the blade of the leaf (2), and the effects of this reaction are expressed in the production of flower primordia at stem growing points often located some distance from the leaf receiving the photoperiodic stimulation. The reduction in extent of floral initiation following application of low night temperatures during treatments with short photoperiod could therefore be considered as an influence of the low temperature on (*a*) the progress of photoperiodic reactions occurring in the leaf blade; (*b*) the transmission of some stimulus from the leaf blade to the terminal meristems of the stem; or (*c*) the differentiation and growth of buds from the terminal meristems. Data dealing with the last two possibilities have been presented previously (4). It was found in that study that floral initiation following a period of treatment with short photoperiods was much less extensive if the low temperatures were restricted either to the petioles of the leaves being subjected to short photoperiods or to the terminal meristems of plants receiving such treatment. The temperature required to reduce materially the amount of floral initiation was found to be about 37°–40°. When temperatures of 45° or higher were employed, very little inhibiting effect was observed. This inhibiting effect of temperatures of 37°–40° on floral initiation was found only when the temperatures were applied continuously, night and day, during the period of treatment with short photoperiods. This was in marked contrast to the results obtained when entire plants received a temperature of 55° during the nights only.

The results of the preceding studies (5, 4) indicated indirectly that inhibition of floral initiation in Biloxi soybean plants following treatments with short photoperiods at low temperature probably resulted from an effect of the low temperature on photoperiodic reactions taking place in the leaf blades. The present paper describes apparatus designed to study this effect and presents data confirming this conclusion.

Material and methods

Each experiment involved the application of several daily 8-hour photoperiods

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[3] Temperatures throughout this paper are Fahrenheit.

and 16-hour dark periods, the latter under conditions of controlled temperature, to a single leaf blade, while all other parts of the plant were exposed to greenhouse temperatures of 70°–75° and to 16-hour or longer photoperiods. Plywood dark chambers were so designed that a single leaf blade of each of twenty plants could be readily inserted or removed without damage to the leaf (fig. 1*B*). Two such boxes, 78 inches long, 25 inches wide, and 33 inches deep, were used simultaneously. The individual leaves were placed inside the box through 3×4-inch holes cut in two sides and one end. Each hole was covered by a 5×6-inch sliding door (fig. 1*A*). The bottom of each door had a narrow slot so cut that when the door was closed over the petiole all but the top end of the slot was covered by the side of the box. The petiole occupied this upper portion so completely that almost no light could enter. Twenty such openings were provided in each box, four on one end and eight on each side. The openings extended about two-thirds of the distance along the sides of the box, leaving about one-third of the box for temperature-control equipment.

The equipment for controlling the temperature within the box consisted of a cooling coil, electric strip heaters, and a fan to circulate the air throughout the box. The temperature of the box was controlled by means of a three-wire bimetallic regulator and mercury relays, the regulator being installed in the part of the box occupied by the leaves. One side of it activated the refrigerant pump whenever the temperature exceeded the regulator setting, and the other side closed a switch in the heating circuit when the temperature fell below the setting. Water was used as the refrigerant, the temperature of which was maintained a few degrees below that desired in the box by a refrigerated constant-temperature bath. With this equipment the temperature during the dark period could be controlled within ±0.5°.

The temperature was recorded continuously by means of a soil thermograph whose bulb was suspended inside the box

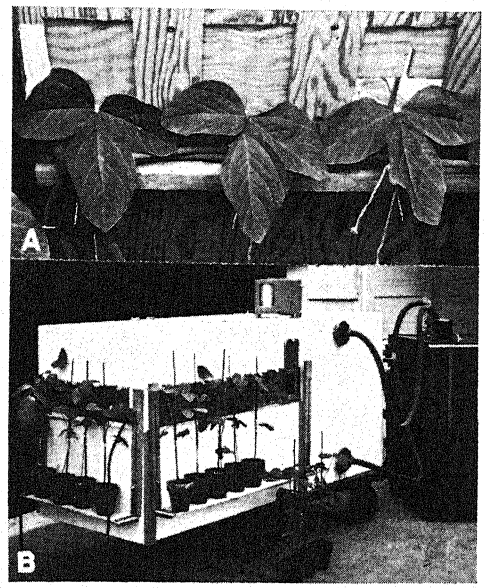


FIG. 1.—Equipment for control of temperature and photoperiod of individual leaf blades: *A*, interior of box showing leaves in position; *B*, exterior of box showing position of plants. Each plant received photoperiodic induction through one leaf inserted in box; rest of plant received long photoperiods and fluctuating greenhouse temperature.

by the side of the thermoregulator. In addition, the temperature in the vicinity of each leaf blade was determined at 8:00 A.M. and 4:00 P.M. each day during the induction treatment, by means of thermocouples. These were installed so that when each leaf was inserted in the box the thermocouple junction was practically touching the lower side of the leaf. The temperature at each of the twenty leaves in the box was determined by means of a portable potentiometer. The continuous thermograph record in-

dicated any irregularities during each experiment, while the thermocouple readings provided a measure of the uniformity of the temperature in the box at any given time.

The plants used were grown in the greenhouse during various times of the year. Both before and after the 5-day induction treatment, all lots received the natural photoperiod, followed by Mazda light of 30–50 foot-candles from sundown until midnight. During the induction treatment the selected leaf was given 8-hour photoperiods in the greenhouse from 8:00 A.M. to 4:00 P.M., whereas all the remaining leaves received light from dawn until midnight.

Five lots of twenty plants each were selected for each experiment from a population of several hundred plants. In this way the plants were closely uniform as to stage of development at the beginning of the experiment.

Two lots of twenty plants each were required for the two boxes and a third lot was used as control at greenhouse temperatures, which usually ranged from 70° to 75° during the night. The plants of this lot were placed on a greenhouse bench near the boxes, and each day during the induction period one leaf blade of each plant was covered with a double-thickness black-cloth bag from 4:00 P.M. to 8:00 A.M. One of the two remaining lots of plants was held continuously on long day and dissected at the end of the experiment; the other lot was dissected the day the experiment was started to determine the total number of nodes in the main stems and to make certain that none of the plants had initiated flower primordia prior to treatment.

Results

The experiments were conducted from December 11, 1941, to October 10, 1942, except for a few weeks during the middle of the summer when temperature control within the boxes was difficult because of excessively high outside temperatures. An attempt was made to repeat the experiments involving similar temperatures at various times throughout the year to avoid faulty conclusions that might result from seasonal variations.

The individual experiments were made with plants of different ages, and the leaf subjected to short photoperiods was also varied, depending on the size of the plant (table 1). In view of these differences in time of year, age of plant, and position of leaf, close agreement of results of individual experiments run at identical temperatures is not expected, because these various factors have been shown to influence the extent of floral initiation resulting from a given photoperiodic-induction treatment (1, 3). Nevertheless, when the data from experiments conducted at identical temperatures are averaged, the trends justify conclusions as to the effect of temperature on the photoperiodic response of a single leaf.

The efficiency of the boxes as dark chambers was tested in two preliminary experiments in which the temperature of the box was the same as that of the greenhouse. A 5-day induction period was given to one group of plants by placing the single leaves in the box and to another group by placing the single leaves in black bags. In each experiment all plants that received photoperiodic induction through the leaf placed in the box initiated flower primordia, as did also most of those that received induction with black bags. The average number of flower primordia per flower-bearing plant was 2.2 for the plants with the single leaf boxed as against 1.6 for the plants with a leaf bagged. These

results indicated that the boxes were entirely satisfactory as dark chambers and that possible light-leaks around the petioles were not causing any reduction in effectiveness of the dark period.

four experiments are not reported in table 1. In three of them none of the plants whose leaf was held at 40° during the dark period formed flower primordia. In the other experiment at 40° one plant

TABLE 1

EFFECT ON FLORAL INITIATION OF VARIOUS TEMPERATURES APPLIED TO SINGLE LEAVES OF BILOXI SOYBEANS DURING 16-HOUR DARK PERIODS OF A 5-DAY INDUCTION TREATMENT. TWENTY PLANTS USED IN EACH TREATMENT OF EVERY EXPERIMENT. EXPERIMENTS CONDUCTED DURING 1942

Experiment no.	Date of start of experiment (1942)	Age of plants (days)	Compound leaf treated	Temperature of boxes (°F.)		Total plants per lot with flower primordia			Total flower primordia per lot		
				Low	High	Low-temperature box	High-temperature box	Controls	Low-temperature box	High-temperature box	Controls
7	1/21	47	4*	45	55	3	14†	20	4	32	79
8	1/26	43	4	45	55	1	15‡	20	2	38	75
33	9/29	31	3	45	55	0	15	19	0	34	73
32	9/21	29	3	50	55	4	12	19	6	36	60
34	10/5	30	3	50	55	3	15	20	3	37	85
9	2/2	49	5	50	60	12	20	20	22	70	99
10	2/9	31	3	50	60	0	5	16	0	6	20
14	3/23	32	2	50	60	7	15	20	14	40	57
15	3/30	40	3	50	60	10	19	20	18	49	75
12	3/9	30	2	55	65	3	14	18	5	28	38
13	3/16	37	3	55	65	6	16	18	10	31	41
16	4/6	32	2	60	70	19	18	20	38	54	64
18	4/20	46	4	70	80	20	20	20	92	87	93
19	4/27	27	2	70	80	20	19	20	80	74	68
20	5/4	34	3	80	90	20	15	18	60	43	53
21	5/11	41	5	80	90	20	20	20	115	114	123
23	5/25	36	4	90	100	20	10	20	87	21	103
24	6/1	29	3	90	105	10	0	20	16	0	69
25	6/8	36	5	90	105	18	2	20	63	3	104
26	6/15	26	2	90	105	15	0	20	31	0	69
27	6/22	33	3	90	105	17	3	19	42	4	58

* Fourth compound leaf from base of plant indicated by 4, third leaf by 3, etc.
† Eighteen plants per lot instead of 20. ‡ Nineteen plants per lot.

The first four experiments were performed with only one temperature control box, which was maintained at 40°, whereas all subsequent experiments were conducted with two boxes. These first

of the twenty formed flower primordia at two nodes. The bagged controls for these experiments averaged two or more flower buds per flower-bearing plant. The long-day control plants of these four experi-

ments and of all others reported in this paper were without flower primordia at the time of dissection.

Three experiments were run in which one box was held at 45° and the other at 55°. The age of the plants varied, and the light intensity during the induction periods was different for the three experiments. All except one plant of the bagged controls in the three experiments initiated flower primordia, the average being 3.8 per flower-bearing plant. The plants that had one leaf at 55° were partially inhibited in their response, since only forty-four of the sixty formed flower buds, and these averaged 2.3 per flower-bearing plant. At 45° the stimulus resulting from short photoperiods was scarcely sufficient to induce the formation of flower buds. In one experiment three plants formed flower primordia, in another only one plant responded, and in the third none formed flower primordia.

In two other experiments the boxes were maintained at 50° and 55°. In both experiments, 50° almost completely inhibited the formation of flower primordia, since they were found in only four plants in one experiment and three plants in the other. At 55° the inhibition was not so marked as at 50°; the response at 55° was similar to that of the first three experiments.

Another series of four experiments was run with the temperature of the boxes maintained at 50° and 60°. In this series the age of the plants varied from 31 to 49 days. The older plants formed more flower primordia in all cases, but the trend toward more flower buds at higher induction temperatures was still evident. At 50° in this series the formation of flower primordia was always less than that at 60°. The small number formed on any of the plants in experiment 10 can be attributed to the fact that the plants were only 31 days old and the light intensity was very low during 2 days of the induction period. Nevertheless the trend shows that 50° definitely reduces the flower-forming stimulus. Induction at 60° also appears to result in some inhibition, because several more plants of the bagged controls held at a greenhouse temperature of 70°–75° initiated flowers than did the plants at 60°, and on the average the control plants produced a few more flower primordia.

In two experiments in which the individual leaves were held at 55° in one box and 65° in the other, the extent of floral initiation at 55° was somewhat less than in certain of the previous experiments conducted at this same temperature. In one experiment, however, the plants were very young and in the other the light intensity during the induction period was unusually low. The plants of experiment 12, although 30 days old, had made slow growth, and the second leaf was used for treatment. In experiment 13 the plants were 37 days old and the third leaf was used for treatment, but the light intensity during the 5-day induction period was very low for the first 3 days and the total for the 5-day period was about half that of the preceding experiment. The trend of the results, however, is the same as that previously reported: 55° inhibited formation more strongly than 65°, and 65° during the dark period reduced the number of flower primordia below that of the bagged controls held at greenhouse temperature.

In another experiment the boxes were maintained at 60° and 70°. The response of the plants to 60° was practically the same as previously reported. The plants whose leaves were held at 70° in the box formed nearly as many flower primordia per flower-bearing plant as did the bagged controls. This was also true for

all the experiments in which temperatures of 70° and 80° were maintained. At 90° under the conditions of these experiments formation of flower primordia was slightly inhibited in comparison with the response of the bagged controls. This inhibition was still more marked at 100°, and at 105° such formation was greatly inhibited in some experiments and completely suppressed in others.

TABLE 2

EFFECT OF TEMPERATURE APPLIED TO SINGLE LEAVES OF BILOXI SOYBEANS DURING 16-HOUR DARK PERIODS OF A 5-DAY INDUCTION TREATMENT

Temperature (° F.)	No. of experiments conducted	Total no. of plants	Total plants forming flower primordia	Percentage of plants forming flower primordia	Total flower-bearing nodes	Total flower-bearing nodes per flower-bearing plant
45	3	60	4	7	6	1.5
50	6	120	36	30	63	1.8
55	7	137	80	58	192	2.4
60	5	100	78	78	203	2.6
65	2	40	30	75	59	2.0
70	3	60	58	97	226	3.9
80	4	80	79	99	336	4.3
90	7	140	115	82	396	3.4
100	1	20	10	50	21	2.1
105	4	80	5	6	7	1.4

In one experiment not reported in the table the temperature was maintained at 110°. This temperature severely damaged the leaf receiving the 16-hour dark period, and all the treated leaves eventually died as a result of the 5-day treatment.

When the results of all the experiments at any one temperature are composited, regardless of the many other factors that differ, the trend of the effects of temperature, applied to a single leaf during the 16-hour dark period, on floral initiation becomes clear (table 2). At 45°, initiation was greatly inhibited, only 7 per cent forming flower primordia. As the temperature to which the single leaf was subjected during the long dark period was increased from 45° to 70°, the percentage of plants forming flower primordia increased. At 70°–90° there was little difference; but at temperatures higher than 90° the formation of flower primordia was definitely less.

Discussion

An earlier paper (4) suggested that the suppressing influence of low temperature on the formation of flower buds on Biloxi soybean plants that had received photoperiodic induction treatment might result from the effects of the low temperature on one or more of three processes. These processes were (*a*) those occurring in the leaf blade which in some way result in the production of a flower-inducing stimulus, (*b*) those concerned with the transmission of this stimulus from the leaf blade to the terminals of the stem where flower buds are formed, and (*c*) those resulting in the actual differentiation and development of the flower primordia at the terminal meristems. In that paper data dealing with the last two processes showed that while floral initiation could be prevented by low temperatures applied either to the petioles of leaves receiving photoperiodic induction or to the terminals of plants whose leaves were receiving such treatments, the temperatures required were relatively low (35°–45°). At 50° very little inhibiting effect was observed.

In the present investigation it was found that temperatures of considerably higher range applied to the leaf blades while they were receiving the long dark periods were effective in suppressing floral initiation. For example, a temperature of 45° applied to the leaf blade almost completely inhibited the formation of flower buds, while a similar temperature applied to the petiole or terminal was previously found to have only a moderately suppressing effect. When the leaf

blades received 55°, about half the plants initiated flower primordia; at 60° and 65° only about three-fourths of them formed flower buds. The data of this and of the preceding paper (4) therefore show that low temperature can influence floral initiation through any of the three processes mentioned, provided it is applied locally to the part concerned.

If low temperature is applied to the entire plant during the dark periods of an induction treatment, however, its inhibiting influence on floral initiation will evidently result primarily from its action on processes occurring in the leaf blade, because as the temperature is lowered from 70° these processes are the first to be suppressed. Consequently, the suppression of translocation of a flower-inducing stimulus or the inhibition of floral differentiation at the terminal meristems resulting from still lower temperatures becomes secondary, since the processes in the leaf that result in the formation of such stimulus have already become completely ineffective. Therefore the results when entire plants were subjected to low night temperature (5) are to be attributed primarily to an effect of low temperature on some chemical process or processes in the leaf blade.

In the present experiments it was found that as temperatures to which the leaf blades were exposed exceeded 90°, there was a decrease in the number of plants forming flower primordia and in the number formed per plant in response to a given photoperiodic induction treatment. Temperatures of this higher range evidently have an inhibiting influence on some process or processes in the leaf blade that are associated with the production of a flower-inducing stimulus. This reduction may come about through a direct effect on the photoperiodic reactions, or it may be the result of the effect of high temperature on secondary reactions not directly associated with flowering. The data do not indicate the reason for this response.

The upper temperature range at which reduced floral initiation became evident is not unusual in the field during the growing season, and the question arises as to whether these high temperatures may not have an inhibiting effect on floral initiation under field conditions. It seems possible that this may occasionally happen, but it is probably not common, since such temperatures seldom occur continuously throughout the night.

It is recognized that the plants were subjected to rather unusual conditions in the experiments involving high temperatures. While the entire plant with the exception of a single leaf received the temperature and humidity prevailing in the greenhouse, this one leaf was subjected to low humidity and high temperature. Although this condition favored high transpiration, the leaves in the box, even at 105°, never showed injury and appeared turgid each morning when they were removed. Nevertheless, such a high transpiration rate in the treated leaf could conceivably have caused inhibition of a flower-inducing stimulus. Such effects, however, have been observed previously when entire plants were subjected to high night temperatures (5). In those experiments the humidity was always moderately high, so it does not seem probable that any unusual conditions of transpiration could have been the causal agent in the present experiments.

Summary

1. Apparatus was devised that made possible the application of controlled temperature to individual leaves of Biloxi soybean plants during the dark periods of a photoperiodic treatment, while

the remainder of the plant received greenhouse temperatures and long photoperiods.

2. When a leaf was held at 50° F. or lower during a 5-day induction period, floral initiation was greatly inhibited. At 70°–90°, such initiation was in general equal to that of the controls held at greenhouse temperatures, but at 90° or higher the extent of the initiation again was less.

3. On the basis of the data presented in this and two preceding papers, the inhibiting effect of low temperature on floral initiation in Biloxi soybean plants appears to be the result of its effect on the photoperiodic reactions occurring in the leaf blade during the dark period, rather than through its effect on translocation of a flower-inducing stimulus from the leaf to the terminal meristems or its effect at the terminal meristems upon the differentiation and development of flower buds.

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LITERATURE CITED

1. BORTHWICK, H. A., and PARKER, M. W., Effectiveness of photoperiodic treatments of plants of different age. BOT. GAZ. 100:245–249. 1938.
2. ———, and ———, Photoperiodic perception in Biloxi soybeans. BOT. GAZ. 100:374–387. 1938.
3. ———, and ———, Floral initiation in Biloxi soybeans as influenced by age and position of leaf receiving photoperiodic treatment. BOT. GAZ. 101:806–817. 1940.
4. BORTHWICK, H. A., PARKER, M. W., and HEINZE, P. H., Influence of localized low temperature on Biloxi soybean during photoperiodic induction. BOT. GAZ. 102:792–800. 1941.
5. PARKER, M. W., and BORTHWICK, H. A., Effect of variations in temperature during photoperiodic induction upon initiation of flower primordia in Biloxi soybean. BOT. GAZ. 101:145–167. 1939.

OCCURRENCE OF ANEUPLOIDS IN LILIUM[1]

ROBERT N. STEWART

Introduction

The genus *Lilium* is favorable material for studies in chromosome morphology, both because of its large chromosomes and because—with the exception of the triploid *L. tigrinum*—all the species reported are diploid, with 24 chromosomes. This would obviously eliminate variations due to chromosome number and thus add significance to species differences in chromosome morphology. During a study of the chromosome morphology and phylogeny of the genus *Lilium* undertaken by the writer, observation of material from approximately ninety species and varieties has revealed the presence of extra chromosomes or fragments in several forms, and a study of their behavior and effect is presented here.

Aneuploid lilies have been reported by SATO (6), who obtained plants with 24–39 chromosomes from selfs and intercrosses of *L. tigrinum*. SATO (5) also reported a *L. japonicum* with 24 chromosomes plus 2 very small fragments. These were found in somatic cells but not in meiosis, and he concluded that they were pieces of the usual 24 chromosomes broken off at constrictions. MATHER (2, 3) reported *L. henryi* with 24 plus 1 fragment and 24 plus 2 fragments. The latter was observed at meiosis, and since the fragments "sometimes paired with each other, sometimes with the long chromosomes and sometimes they were unpaired," he concluded "that they were alike, were reduplicated segments of the large chromosomes, and were not entirely homologous with any piece of any one chromosome, but were made up of a small number (about three) of pieces, each homologous with different sections of the normal complement." MATHER also examined meiosis in a *L. japonicum* with 24 chromosomes plus 2 fragments. These were described as like those of *L. henryi*, except that they were of two sizes, "the larger one appearing to consist of a piece like the smaller one, with another small section joined to it." He continued, "Since, however, SATO (1932) found the same number of fragments in *L. japonicum* as I did, it would appear that this is a clonal species." SANSOME and LA COUR (4) state that "*L. japonicum* has one or possibly two fragments." A list of chromosome numbers they obtained from root tips includes the following two, but there is no discussion of them in the text: "*formosanum*. . . . 24 (+2ff?)" and "*tigrinum splendens*. . . . 36 (+f?)." BEAL (1) found a fragment in plants of *L. willmottiae* in addition to the 12 normal bivalents. This fragment never pairs with any of the major chromosomes. It moves to one pole or the other at anaphase I, but its behavior in meiosis II has not been determined. When crosssed with *L. davidii*, a species in which BEAL found no fragment, one of the hybrids had no fragments, ten had one fragment, and one had two fragments, of which one was the size of that in *L. willmottiae* while the other was smaller.

The lilies used in the present studies were all obtained from commercial dealers in the United States and Canada, many of the species being represented by several plants from each of two or more dealers. Buds were killed in 3:1 absolute alcohol–glacial acetic acid for 24 hours and then stored in 80 per cent alcohol. Root tips were treated in 1 per cent colchicine solution 1½ hours before killing (7). Smears of both buds and roots were

[1] Scientific article no. A55, Contribution no. 1890, Maryland Agricultural Experiment Station (Botany Department).

made by the usual propiono-carmine method.

Results

LILIUM PUMILUM (L. TENUIFOLIUM)

Chromosome counts were made in fourteen plants of *L. pumilum* from five different sources, twenty-five plants of *L. pumilum* var. Golden Gleam from four sources, and four plants of *L. pumilum* var. Red Star from a single dealer. This species is generally increased by seed, and it is known for certain that at least two of the dealers used that practice, so the plants are individuals and not vegetatively reproduced from single bulbs. In obtaining the somatic chromosome counts in this and the following species, camera-lucida drawings of each chromosome were made, the chromosomes paired, and idiograms constructed as described earlier (7). The morphology of the basic complement of 12 pairs of chromosomes was identical in all three forms.

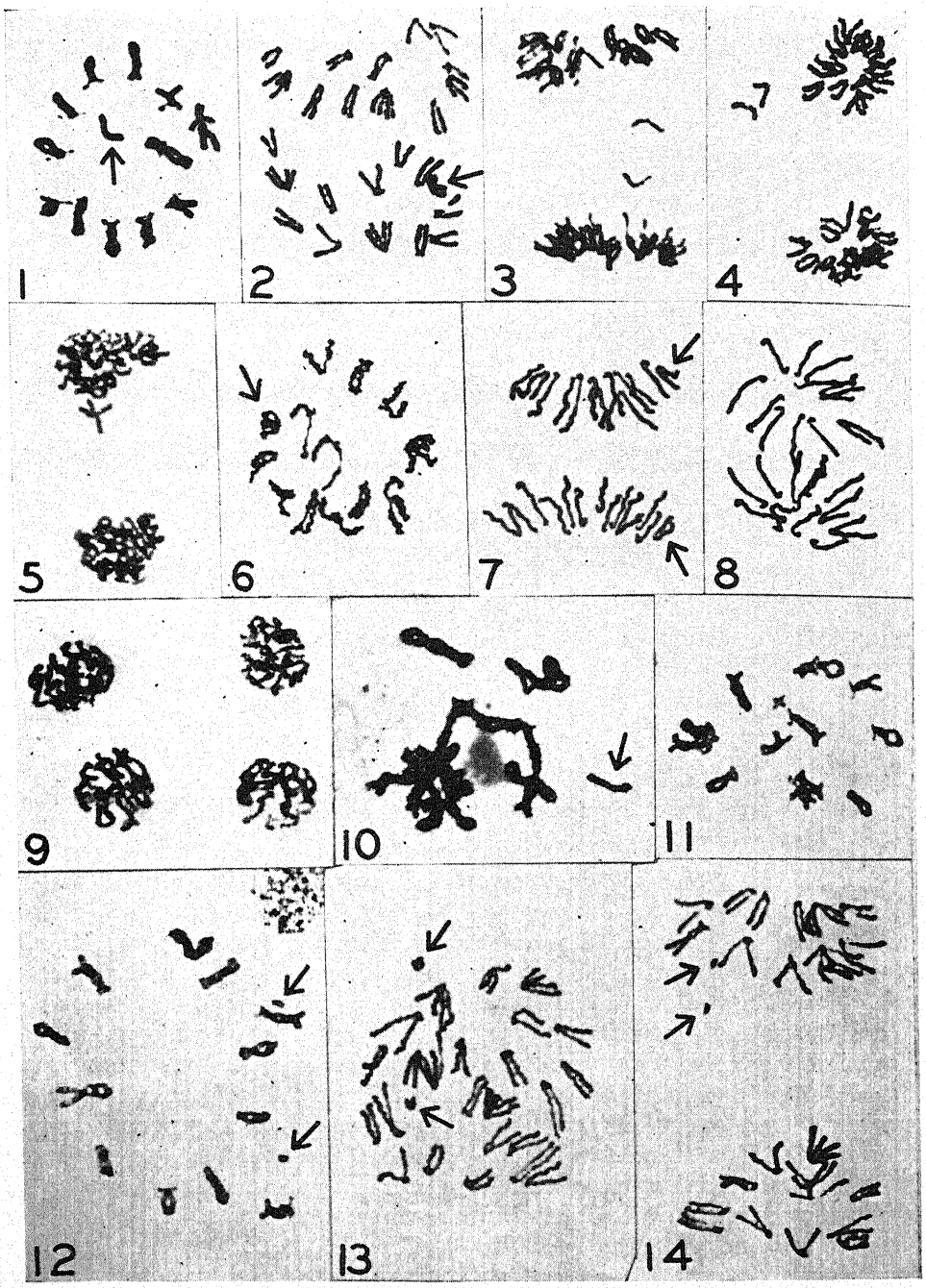
Of the fourteen plants of *L. pumilum*, nine had 24 chromosomes while the other five each had 25. The extra chromosome was the same in each case. This same chromosome was present in nine of the twenty-five plants of *L. pumilum* var. Golden Gleam but in none of the four of var. Red Star. The three forms have, like all other diploid *Lilium* species examined, two pairs of very long chromosomes with submedian kinetochores and 10 pairs of progressively shorter chromosomes with subterminal kinetochores (figs. 7, 8, 22, 23). The morphology of the extra chromosome was different from all others in that it had a median kinetochore, each arm being about the same length as the shorter arm of the chromosomes with submedian kinetochores (fig. 7).

In meiosis I, pairing was always complete in the 24-chromosome plants, but in the 25-chromosome plants there were always 12 bivalents and 1 univalent (fig. 1), the extra chromosome showing no pairing in more than 5,000 cells at diakinesis or metaphase I. In cells which were not flattened, the side view showed that it was seldom in the equatorial plate but was near one of the poles. In more than 90 per cent of the cells at anaphase I the univalent moved to a pole without splitting (fig. 2), the chromatids separated except at the kinetochore and were still tightly coiled. When it was located on the equatorial plate at metaphase I, the univalent lagged and split, the halves usually going to opposite poles (fig. 3) but occasionally to the same pole (fig. 4). At telophase I the complete univalents were sometimes at the edge of the nuclear mass (fig. 5), but then at metaphase II it was included on the plate (fig. 6) and separated normally at anaphase (fig. 7). The other nucleus of the dyad showed only the normal complement of 12 chromosomes at each pole (fig. 8). In more than 1,500 cells observed in meiosis II, no trace of split univalents—such as would result from the behavior shown in figures 3 and 4—was found. In the tetrad stage (fig. 9) not one in the thousands of cells observed had any micronuclei.

The presence of this chromosome could be detected without examining the chromosomes, as it produced a pronounced dwarfing, the 25-chromosome plants being about half the height of the 24-chromosome plants.

Three of the *L. pumilum* var. Red Star plants had 24 chromosomes, but the fourth had 26. The extra pair were alike in morphology, being very short with subterminal kinetochores.

At metaphase of meiosis I, the extra chromosomes either paired with each other (fig. 11) or were present as 2 uni-



FIGS. 1–14.—Microsporogenesis in *Lilium*. Arrows point to some extra chromosomes. Figs. 1–10, *L. pumilum* var. Golden Gleam (1, metaphase of 25-chromosome plant showing 12 bivalents and extra chromosome as univalent; 2, anaphase I, univalent moving to pole without splitting; 3, anaphase I, univalent split and halves moving toward opposite poles; 4, anaphase I, univalent split and halves moving toward same pole; 5, telophase I, complete univalent excluded from nucleus; 6, metaphase II, 13 chromosomes; 7, anaphase II, 13 chromosomes at each pole; 8, anaphase II, 12 chromosomes at each pole; 9, tetrad stage, no chromosomes excluded; 10 [*L. tsingtauense*], diakinesis showing 12 bivalents and 1 univalent). Figs. 11–14, *L. pumilum* var. Red Star (11, metaphase I, extra chromosomes paired; 12, metaphase I, extra chromosomes as univalents; 13, anaphase I, normal separation of extra chromosomes after pairing at metaphase; 14, anaphase I, both univalents moving to same pole).

valents (fig. 12), but they never paired with any of the normal complement of chromosomes. When the 2 extra chromosomes were paired, they separated normally at the first division (fig. 13) and in the second, giving 4 microspores, each with the extra chromosome. When the extra chromosomes were unpaired, they both moved without splitting, either to the same pole (fig. 14) or to different poles. In some cells both univalents split, the halves going to opposite poles (fig. 15), and in others 1 univalent split, with the halves going to opposite poles and the other univalent moving to a pole without splitting (fig. 16). The second division results in microspores with 0, 1, or 2 of the extra chromosomes, as none lag enough to be excluded from the microspore nuclei. The extra chromosomes had no marked phenotypic effect.

Lilium tsingtauense

Only one plant of this species was available. Examination of root-tip cells in the division showed 25 chromosomes. There were the usual 12 pairs plus an extra one, which had a subterminal kinetochore and was slightly shorter than the shortest of the 10 pairs with subterminal kinetochores.

Only one bud was available for study of meiosis. The microspore mother cells were in diakinesis I and metaphase I, always showing 12 bivalents and 1 univalent (fig. 10), in this respect being similar to the 25-chromosome plants in *L. pumilum* and *L. pumilum* var. Golden Gleam.

Lilium japonicum

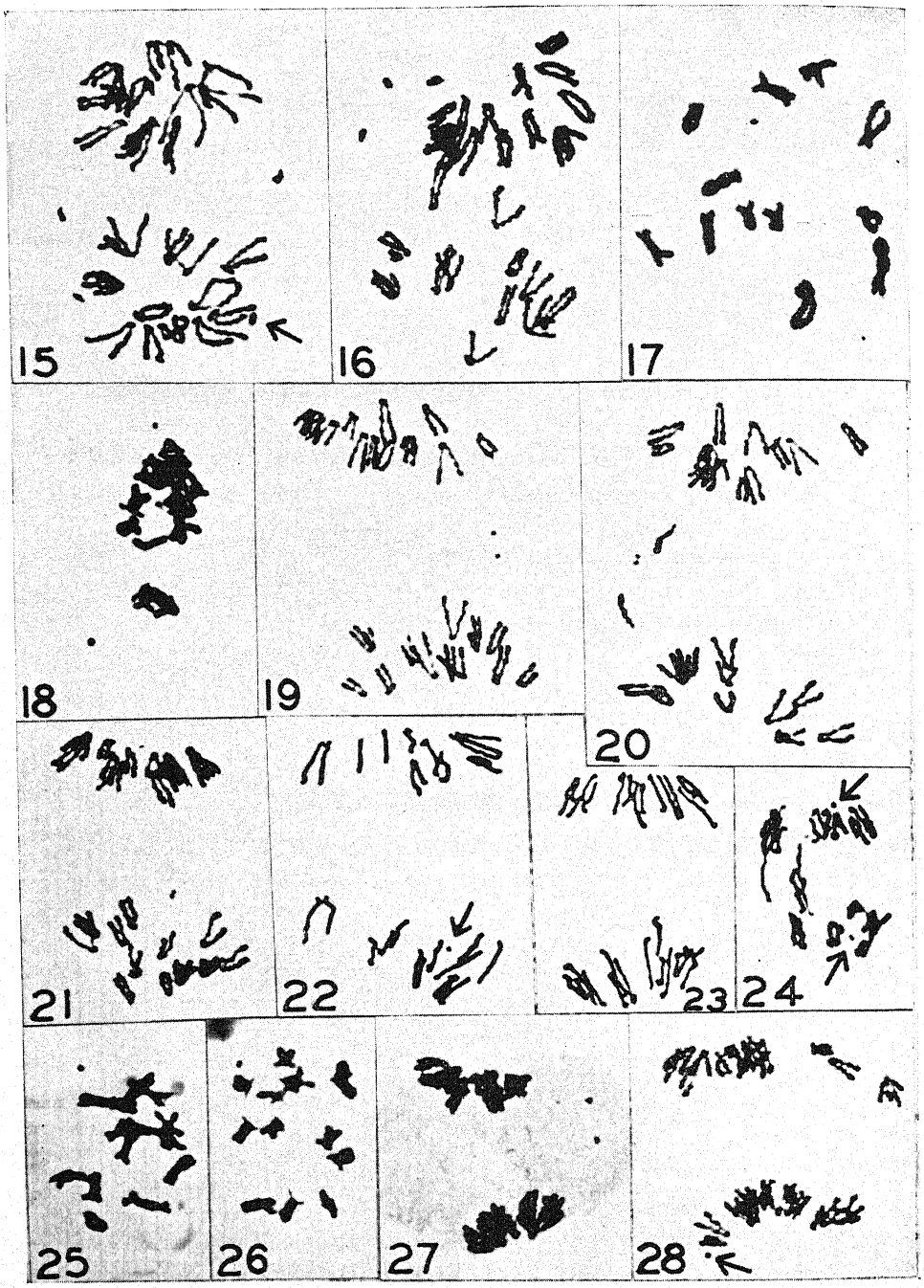
Two plants from one dealer were examined. One had 24 chromosomes, and the other in addition had 1 very small centric fragment both in the root tips and in buds. Occasional univalents were found in the normal complement at metaphase of meiosis I, and the fragment was never paired (fig. 17). One to 2 per cent of the cells at this stage had 1–3 additional fragments of varying size (fig. 18), but these were not seen at later stages. At anaphase I the fragment sometimes lagged and divided (fig. 20), the halves going to opposite poles (fig. 19), and sometimes moved to one pole or the other without splitting (fig. 21). At anaphase II two types of distribution were found, 12+f-12 with 12+f-12 (fig. 22) and 12-12 with 12+f-12+f (figs. 23, 24). The fragments were always included in the microspore nuclei. No marked phenotypic effect of the fragment was observed.

Lilium canadense

The root tips of seventeen plants from four sources all showed 12 pairs of chromosomes. Meiosis in sixteen plants showed 12 bivalents at diakinesis and metaphase, but the other plant had in addition a small fragment which never paired with any normal complement (figs. 25, 26). This fragment divided in the first division in a few of the cells (fig. 27) but usually moved to a pole without splitting (fig. 28). Later stages were not present in the two buds from this plant. Additional roots showed no fragment present. No marked phenotypic effect of the fragment was evident.

Lilium batemanniae

The root tips of three plants from two dealers were examined. Two plants had 12 pairs of chromosomes, but one of the two from a single source had in addition a twenty-fifth chromosome possessing a subterminal kinetochore and being slightly shorter than the shortest of the 10 pairs with subterminal kinetochores. No bud material was collected. There was no marked phenotypic difference be-



FIGS. 15–28.—Microsporogenesis in *Lilium*. Arrows point to some extra chromosomes. Figs. 15, 16, *L. pumilum* var. Red Star (15, anaphase I, 2 univalents split with halves separating; 16, anaphase I, 2 univalents, 1 split and other moving to pole without splitting). Figs. 17–24, *L. japonicum* (17, metaphase I, 12 bivalents and univalent centric fragment; 18, metaphase I, 2 fragments; 19, anaphase I, fragment lagging and split, halves going to opposite poles; 20, anaphase I, univalent of normal complement and fragment lagging and splitting; 21, anaphase I, fragment univalent moving to pole without splitting; 22, anaphase II, 12-12+f distribution; 23, anaphase II, 12-12 distribution; 24, anaphase II, 12+f-12+f distribution). Figs. 25–28, *L. canadense* (25, diakinesis, 12 bivalents and fragment; 26, metaphase I, 12 bivalents and fragment; 27, anaphase I, fragment split with halves moving to opposite poles; 28, anaphase I, univalent at pole without splitting).

tween the plants with the extra chromosome and those lacking it.

Lilium willmottiae

Three plants from one source had 24 chromosomes in root tips and buds. One plant from another source carried in addition a small centric fragment both in root tips and buds. Its behavior in meiosis was similar to that of the fragment in *L. japonicum*. The fragment produced no marked phenotypic effect.

Lilium formosanum, L. tigrinum splendens, L. henryi, and others

Study of somatic chromosome morphology and of meiosis in three forms of *L. formosanum*, four collections of *L. henryi*, numerous plants of *L. tigrinum splendens*, and approximately eighty other species and varieties have revealed no fragments in addition to the complement of 24 chromosomes in the diploids and 36 in the triploid forms of *L. tigrinum*.

Discussion

The most interesting feature of the extra chromosomes and centric fragments reported here is the fact that they never pair with any of the normal complement of chromosomes, even at the earliest stages in which pairing could be seen. This complete lack of association and chiasmata formation with the basic complement of 12 pairs of chromosomes indicates that there are no blocks of homologous genes in the fragment large enough to allow any crossing-over (chiasma formation). If the extra chromosomes were duplications of blocks of material in the basic complement, although the amount of pairing would be reduced by competition with the normal homologue, nevertheless there would be some crossing-over.

There is no evidence to suggest that the species containing the extra chromosomes and fragments are hybrids. The somatic chromosomes in each case could be arranged into 12 homomorphic pairs. While a few bridges and fragments were found at meiosis in these species, indicating the presence of heterozygous inversions, these configurations were no more frequent than in any other of the "good" species of *Lilium*.

These two features, and the lack of other evidence, make it impossible to determine the origin of these chromosomes. The asexual reproduction in *Lilium* would tend to preserve the aneuploid numbers. Cytological evidence has shown that the extra chromosomes are always included in the male gametes. The fact that some of the plants of *L. pumilum* and *L. pumilum* var. Golden Gleam used in the present study carried an extra chromosome indicates that they will pass through the male and/or female gamete. Failure to find 2 extra chromosomes in one of these plants might be due to decreased viability or to failure of extra chromosome gametes to function on the male or the female side. Seeds have been obtained on selfing to test the inheritance of the extra chromosomes.

The dwarfing associated with the extra chromosome in *L. pumilum* and *L. pumilum* var. Golden Gleam indicates that it is genetically active. More extensive observation of the plants with extra chromosomes in the other species would probably reveal less marked phenotypic effects. However, no evidence was found regarding the origin or the possible phylogenetic significance of the extra chromosomes and centric fragments.

Summary

Counts of 25 and 26 chromosomes were made in plants of *L. pumilum*, *L. pumilum* var. Golden Gleam, *L. pumilum* var.

Red Star, *L. tsingtauense*, *L. japonicum*, *L. canadense*, *L. batemanniae*, and *L. willmottiae*. Approximately eighty other species and varieties showed no extra chromosomes present. The morphology of the extra chromosomes was in all cases different from that of the normal 24 chromosomes, and in meiosis they never paired with the normal complement. At least one of the extra chromosomes contains some active genic material.

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LITERATURE CITED

1. BEAL, J. M., Chromosome fragments in *Lilium willmottiae* and hybrids between it and *L. davidii*. BOT. GAZ. 103:617–619. 1942.
2. MATHER, K., The chromosomes of *Lilium*. II. R.H.S. Lily Yearbook 3:38–40. 1934.
3. ———, Meiosis in *Lilium*. Cytologia 6:354–380. 1935.
4. SANSOME, E. R., and LA COUR, L., The chromosomes of *Lilium*. III. R.H.S. Lily Yearbook 3: 40–45. 1934.
5. SATO, M., Chromosome studies in *Lilium*. I. Bot. Mag. Tokyo 46:66–88. 1932.
6. ———, Chromosome variations in the progeny of triploid *Lilium tigrinum*. Cytologia Fujii Jub. Vol. 1056–1061. 1937.
7. STEWART, R. N., and BAMFORD, R., The nature of polyploidy in *Lilium tigrinum*. Amer. Jour. Bot. 30:1–7. 1943.

CYTOLOGY OF THE AUTOTETRAPLOID RYE[1]

T. C. CHIN

Introduction

Although in recent years colchicine has been used extensively in the induction of polyploid plants, its successful application to cereal crops has been comparatively rare.

Autotetraploid rye has been obtained by DORSEY (2) by high temperature treatment after pollination, but unfortunately no detailed cytological work was included. In 1940 Dr. H. W. LI obtained an autotetraploid rye (*Secale cereale*) by immersing the ears in an aqueous solution of colchicine during meiosis. The writer secured some of the seeds from Dr. LI, to whom he is much indebted. The smears were stained by aceto-carmine. The pollen grains and the pollen tubes were fixed by Watkins' cotton blue.

Investigation

Generally the plants of autotetraploid rye are taller, the ears are longer, and the flowers and the seeds all bigger in comparison with its diploid progenitor. The size of the cells is greater. Measurements of the stomata (fig. 1) and the pollen grains (fig. 2) are listed in table 1. It will

TABLE 1

SIZE OF CELLS OF DIPLOID AND TETRAPLOID RYE

RYE	LENGTH OF GUARD CELLS OF STOMATA	NO. OF STOMATA PER 0.01188 SQ. MM. OF LEAF			SIZE OF POLLEN GRAINS
		Upper surface	Lower surface	Ratio lower/upper	
Diploid....	2.56	86.5	68.5	0.79	3.14×2.18
Tetraploid..	3.26	50.0	46.5	0.93	3.72×2.98

be seen that the number of cells per unit area is comparatively less in the autotetraploid than in the diploid, owing to the greater size of the cells in the former.

[1] Contribution from the Department of Agronomy, College of Agriculture, University of Nanking.

MEIOSIS

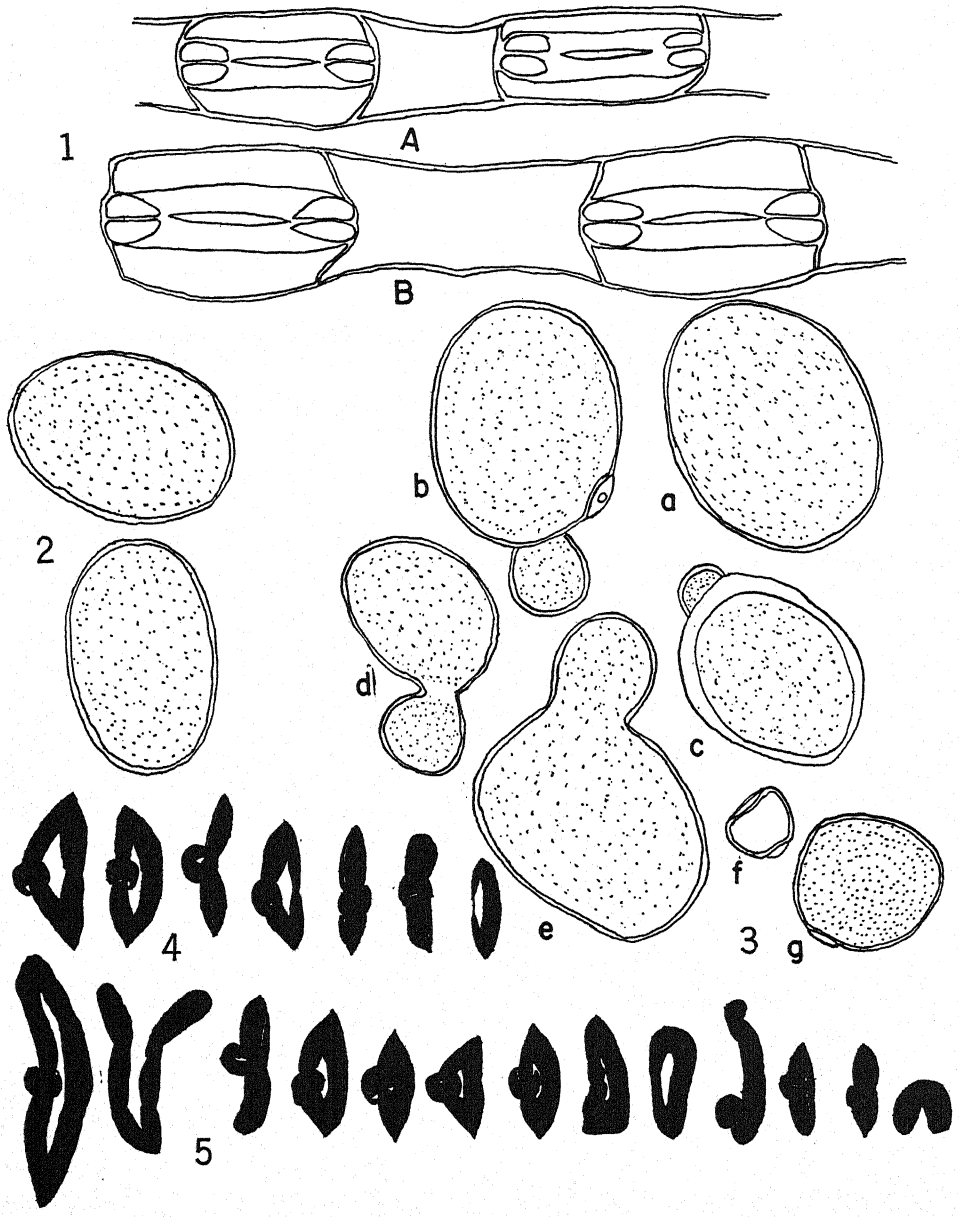
METAPHASE CONFIGURATIONS. — The diploid shows invariably 7 bivalents (fig. 4). Only rarely can two univalents be found. The meiotic division of the autotetraploid (fig. 5) shows great variations. The maximum number of multivalents is 3, and the maximum number of univalents is 5. The combinations of the configurations are given in table 2.

TABLE 2

COMBINATIONS OF CONFIGURATIONS OF TETRAPLOID RYE

Configurations	Frequency
3(IV)+8(II)	1
2(IV)+10(II)	2
2(III)+10(II)+2(I)	1
1(IV)+1(III)+8(II)+5(I)	1
1(IV)+1(III)+10(II)+1(I)	1
1(IV)+12(II)	4
1(IV)+11(II)+2(I)	2
1(III)+12(II)+1(I)	2
1(III)+11(II)+3(I)	1
14(II)	3
12(II)+4(I)	1
13(II)+2(I)	2
Total no. of cells	21

The average number of multivalents per cell is 1.05. Six out of twenty-one cells (28.6 per cent) possess no multivalents. The maximum number of chromosomes forming quadrivalents is 12/28, or 42.9 per cent. The proportion of chromosomes forming multivalents varies with organisms. Thus MÜNTZING (6) classified the autopolyploids into two main categories: (*a*) organisms wherein all the chromosomes may form multivalents, with a variable number of other configurations in different cells; (*b*) organisms wherein only a very low percentage of chromosomes form multivalents. The writer's results on the autotetraploid rye in comparison with his previous results



FIGS. 1–5.—Fig. 1*A*, stomata of diploid rye; fig. 1*B*, of tetraploid rye. Fig. 2, haploid pollen from diploid rye. Fig. 3, diploid pollen from tetraploid rye (*a*, normal pollen; *b–e*, lobed pollen; *f*, small empty pollen; *g*, small pollen). Fig. 4, configurations of first metaphase of 2n rye. Fig. 5, same of 4n rye.

on the autotetraploid barley are shown in table 3.

The difference in the frequencies of the multivalents is due to the length of the chromosomes (3, 8) and possibly also to their structure. Regarding their length, the writer found that the multivalents are almost all composed of the longer number of multivalents in barley. Probably the relative lengths of the chromosome arms as well as their total lengths are the determining factors (4). No multivalents other than rings or chains were found in the 4n rye.

Tables 4 and 5 give the distribution of the chiasmata for each individual cell.

TABLE 3

DISTRIBUTION OF MULTIVALENTS IN TETRAPLOID RYE AND BARLEY

No. of multivalents per cell (4n=28)	0	1	2	3	4	5	6	7
Barley*	0	1	2	3	6	4	4	1
Chin (previous publication)	0.0	4.3	9.5	14.3	28.6	19.0	19.0	4.8
Rye†	6	9	5	1				
Chin (present paper)	28.6	42.9	23.8	4.7				

* No. of cells observed, 21; mean no. of multivalents, 4.24.

† No. of cells observed, 21; mean no. of multivalents, 1.05.

TABLE 4

DISTRIBUTION OF CHIASMATA IN AUTOTETRAPLOID RYE*

No. of chiasmata	25	26	27	28	29	30	31	32	33	34	35.....39
Frequency	1	1	0	3	3	2	2	1	1	5	1..... 1

* No. of cells observed, 21; mean no. of chiasmata per cell, 31.10.

TABLE 5

DISTRIBUTION OF CHIASMATA IN DIPLOID RYE*

No. of chiasmata	12	13	14	15	16	17	18	19	20
Frequency	1	0	2	0	1	3	2	2	2

* No. of cells observed, 13; mean no. of chiasmata per cell, 17.00.

chromosomes in the 4n rye, and this, according to UPCOTT (8), is due to the possible frequency of chiasma formation. The percentage of chromosomes forming multivalents seems to be determined by the size and the possible chiasma frequency of the multivalent components and not by the total chiasma frequency of each cell. Thus in barley (*Hordeum bulbosum*), which possesses an average number of quadrivalents of 4.24 per cell, the total chiasma frequency is 25.62 in comparison with 31.10 in the autotetraploid rye, of which the average number of multivalents (quadrivalents and trivalents) is only 1.05; and even the maximum in the 4n rye does not exceed the mean

The mean numbers of chiasmata per cell are 31.10 for the tetraploid and 17.00 for the diploid rye. The reduction factor in the chiasma frequency of the autotetraploid is 0.9118. This reduction is due, according to UPCOTT (9), to the greater size of cells of the polyploid plants; consequently a delay in pairing results in a reduction of chiasma frequency. The writer's results confirm this finding. The chiasma frequency, even when multivalents are absent, is always lower than twice the chiasma frequency of the diploid. The average number of chiasmata of these six cells, wherein no multivalents are found, is 32.3. The reduction factor is 0.95.

A comparison of half chiasmata per chromosome between the diploid and the tetraploid is shown in table 6 and figure 6. The autotetraploid shows a lower number of half chiasmata for each chromosome. The distribution for the autotetraploid shows a bimodal curve, although the general trend for the two is similar, with the exception of the location of the mode.

TABLE 6

DISTRIBUTION OF HALF CHIASMATA PER CHROMOSOME IN RYE

No. of half chiasmata...	0	1	1.3	1.5	2	2.5	3	4	
Diploid.....	6	16	.....	.....	70	.....	86	4	(Mean 2.36)
	(3.3)*	(8.8)	.....	.....	(38.5)	.....	(47.3)	(2.2)	.....
Tetraploid.....	25	26	18	28	281	4	200	6	(Mean 2.10)
	(4.3)	(4.2)	(3.1)	(4.8)	(47.8)	(0.7)	(34.0)	(1.0)	

* Percentages in parentheses.

TERMINALIZATION OF CHIASMATA.—The average number of terminal chiasmata is 11.85 for the diploid and 24.76 for the autotetraploid; the coefficients of terminalization are 0.6971 and 0.7987, respectively. The higher degree of terminalization in the autotetraploid is due to the fact that the majority of the multivalents possess terminal chiasmata only.

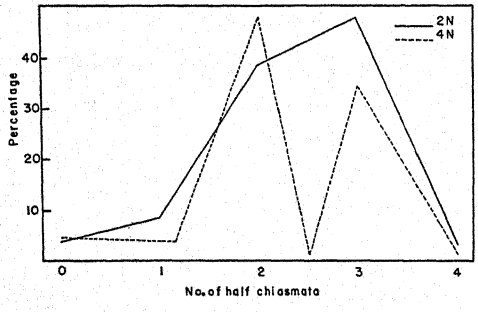


FIG. 6

INVERSION.—Bridges (figs. 7, 8) have been observed in the first anaphase; the second anaphase is normal. The total of pollen mother cells possessing a single bridge in the first division is 7.0886 per cent. The coefficient of hybridity is 0.00229. The maintenance of such a heterozygosity is due to cross pollination.

The coefficient of hybridity is not high enough to account for the reduction in the percentage of chromosomes forming multivalents, since in barley the coefficient of hybridity, amounting to 0.004 (1), is nearly 1.8 times as high as that of the autotetraploid rye. However, structural changes of this sort will eventually lead to a reduction in the chiasma frequency, and consequently the formation of multivalents is avoided (9). Then the organism will no longer be an autotetraploid but an allotetraploid.

LAGGARDS.—The laggards usually divide in the first and lag in the second division. Those which are excluded from the spindle of the first division may form microcytes (fig. 9) in the second.

GAMETE FORMATION AND STERILITY

There are two kinds of abnormal pollen grains: (*a*) abortive ones of varying size, the percentage of such grains being 2.3; (*b*) lobed grains (fig. 3). The first leads to partial sterility. The average seed setting is 23.8 per cent, ranging from 7.8 to 34.5 per cent in comparison with about 75 per cent of fertility in the diploid. Evidently the abortion of pollen grains is not the only cause of the partial sterility in the tetraploid. In addition, MÜNTZING (6) described two other causes—diplontic and haplontic sterility. The lobed pollen grains may be binucle-

ate (5, 7, 9). The main body of the grains is on the whole smaller than that of the normal ones. The size of the lobe is negatively correlated with that of the main bodies. The number of such pollen grains is 0.8 per cent. The occurrence of small pollen grains (fig. 3*f*, *g*) shows that the lobed ones owe their origin to the failure of cell-wall formation. There is no evidence to show what number of chromosomes the lobed grains may possess and even to what degree they are fertile.

Incompatibility

Cross pollination shows that only 4n × 2n (fig. 12) would result in normal seed setting, and 2n × 4n (fig. 13) is entirely incompatible. Investigation of the pollen-tube growth shows that it is simply due to the interference of the 2n growth in the 2n style (table 7).

TABLE 7

POLLEN-TUBE GROWTH AND SEED SETTING

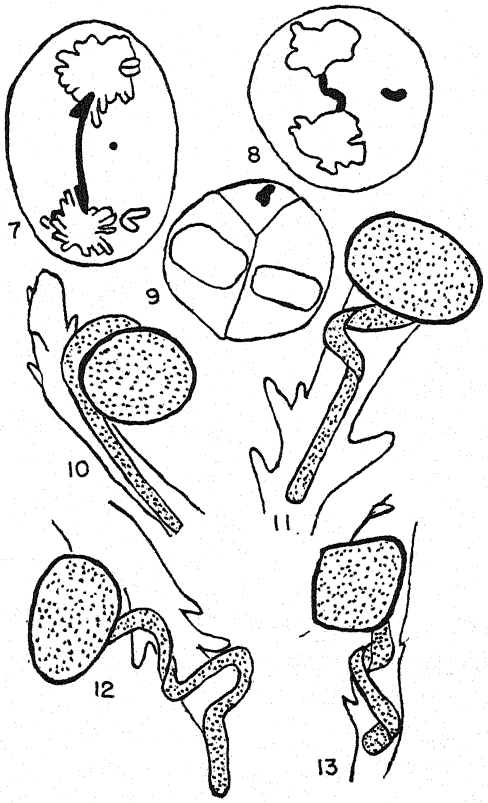
Plants	Pollen germinated (%)	Period after pollination (hours and minutes)	Length of tube growth per hour (μ)	Seed setting (%)
2n selfed....	12.4	6:30	12.24	.....
4n selfed....	8.5	5:50	12.08	33.3
4n × 2n.....	10.0	5:55	11.60	38.2
2n × 4n.....	2.3	6:55	3.0 *	0.0
2n × 4n.....	5.0	22:05		0.0

* Total length of tube was 19.00 μ for 22 hours and 50 minutes after pollination, in comparison with 17.00 μ for 6 hours and 55 minutes.

This agrees with Watkins' (10) statement that "whereas if the tissue number be less than double that of the pollen (e.g., 2X pollen tube in 2X styles), pollen tube growth will be seriously interfered with." The difference in the behavior is due to the difference in the osmotic pressure, since the 2n pollen grains may shrink on the 2n styles.

Summary

1. The autotetraploid rye (*Secale cereale*) shows gigantism in cell size as well as in the plant as a whole.



Figs. 7–13.—Figs. 7, 8, bridge and fragment of first anaphase of 4n rye. Fig. 9, laggard and the microcyte. Fig. 10, tube of haploid pollen on diploid style, 6 hours and 30 minutes after pollination. Fig. 11, tube of diploid pollen on tetraploid style, 5 hours and 50 minutes after pollination. Fig. 12, tube of haploid pollen on tetraploid style, 5 hours and 55 minutes after pollination. Fig. 13, tube of diploid pollen on diploid style, 22 hours and 5 minutes after pollination.

2. The meiotic metaphase shows a maximum number of quadrivalents of 4, and the mean number per cell is 1.05. No multivalents were found in 28.6 per cent of the cells.

3. Chiasma frequency of the autotetraploid rye in comparison with that of the diploid is discussed.

4. Bridges of the first division have been observed. The coefficient of hybridity is 0.00229.

5. Lobed pollen grains have been found, and this is ascribed to failure of cell-wall formation. On the average, the seed setting is 23.8 per cent.

6. Cross pollination shows that only 4n × 2n sets seed and that 2n × 4n is entirely incompatible, owing to the failure of pollen-tube growth.

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LITERATURE CITED

1. CHIN, T. C., The cytology of some wild species of *Hordeum*. Ann. Bot. n.s. 535–545. 1941.
2. DORSEY, E., Induced polyploidy in wheat and rye. Jour. Hered. **27**:155–160. 1936.
3. KLINGSTEDT, H., On some tetraploid spermatocytes in *Chrysochraon dispar* (Orth.). Mem. Soc. Fauna Flora Fenn. **12**:194–209. 1937.
4. MATHER, K., The determination of position in crossing-over. III. The evidence of metaphase chiasmata. Jour. Genet. **39**:205–223. 1940.
5. MOFFETT, A. A., Studies in the formation of multinuclear giant pollen grains in *Kniphofia*. Jour. Genet. **25**:315–337. 1932.
6. MÜNTZING, A., The evolutionary significance of autopolyploidy. Hereditas **21**:263–378. 1936.
7. RAMANUJAM, S., Cytogenetical studies in the Oryzeae. III. Cytogentical behavior of an interspecific hybrid in *Oryza*. Jour. Genet. **35**: 223–258. 1938.
8. UPCOTT, M., The genetic structure of *Tulipa*. III. Meiosis in polyploids. Jour. Genet. **37**:303–339. 1939.
9. ———, The nature of tetraploidy in *Primula kewensis*. Jour. Genet. **39**:79–100. 1939.
10. WATKINS, A. E., Hybrid sterility and incompatibility. Jour. Genet. **25**:125–162. 1932.

ROLE OF PYRENOIDS IN ALGAE AND OF VACUOLES IN PLASTIDS OF HIGHER PLANTS AND IN FUNGI

S. R. BOSE

Introduction

Little is known with regard to the exact function of pyrenoids in algae. According to SMITH (11), the pyrenoid synthesizes starch. When cells are grown in pure culture in total darkness but in the presence of glucose or some other simple carbohydrate, starch continues to accumulate as a series of grains around the pyrenoid. In certain of the Rhodophyceae, however, starch appears outside the plastid. In *Oedogonium*, for example, besides the pyrenoid starch there is stroma starch in the body of the sub-parallel meshes of the reticulate chloroplast. CLELAND (4) reports a close association of the pyrenoids of *Nemalion* with the formation of floridean starch, which is held to be a compound intermediate between true starch and dextrin.

In diatoms, where there is no starch sheath around the pyrenoid, SMITH (12) states that the pyrenoids possibly function as elaioplasts and may be concerned in the formation of oil. In this connection KARSTEN (7) reports that the role which pyrenoids play in starch-free diatoms remains unknown; apparently it is a constructive role in the metabolism of CO_2 assimilation, although the fatty oil in relation to pyrenoids has not definitely been observed.

Pyrenoids are probably not reserve proteins, as several are found in the youngest cells of *Oedogonium* and *Spirogyra*. Also, there are many algae (Siphonales, Myxophyceae, Charales, etc.) which lack pyrenoids but can produce starch, fat, glycogen, and sometimes even protein as reserve substances.

Pyrenoids would seem to be related to nutrition, since they become prominent during periods of abundant food supply and apparently disappear during periods of starvation. STEINECKE and ZIGENSPECK (14) consider that pyrenoids constitute receptacles of a starch-building ferment, but their experiments on changes of pyrenoids during starch production with *Spirogyra grevilleana* and *Zygnema stellinum* do not support their contention.

The present investigations were undertaken to elucidate the role of pyrenoids in algae. Observations were also made on vacuoles in fungi and in plastids of higher plants. A preliminary note on the subject has been published (3).

Observations

There is usually the synthesis of starch around the pyrenoids in green algae. In *Oedogonium* the stroma starch is deposited around the vacuoles in the body of the reticulate chloroplast and the pyrenoid starch is found around the pyrenoids, which show vacuoles at their center. In the case of starch-free diatoms, the first formation of oil takes place around the pyrenoids (fig. 1).

A faint pinkish stain is found in the pyrenoids at their center; this is true not only in cases of pyrenoid-bearing Chlorophyceae and Diatomaceae, but also in cases of *Anthoceros* and *Selaginella*, where—especially in *Anthoceros*—the "pyrenoids consist of a crowded mass of 25 to 300 disk- or spindle-shaped bodies, each of which may be transformed into a rudimentary starch grain without changing its form or position" (13). The same pinkish vacuolar stain is found in the chloroplasts of higher plants as well as of the Chlorophyceae without pyrenoids (*Vaucheria*, *Chara*, etc). In the Myxophyceae, which have no pyrenoids

and where reserve substances are usually fat and glycogen, pinkish vacuoles have been found, usually one in each cell, and oil globules are grouped around the single vacuole of each cell of the trichome.

Regarding the structure of chloroplasts in higher plants, ZIRKLE (15) states that one or more "cavities" are present in chloroplasts before starch appears in them. A faint pinkish stain is found in these cavities, and starch accumulates in the periphery of the plastid with the central vacuole. This stain is not found in old, disorganized or plasmolyzed cells of *Spirogyra*, etc. The same stain has been found (1, 2) in vacuoles of growing hyphae of diverse groups of fungi, evidently connected with the general metabolism of these fungi. From these observations the writer concludes that the vacuole probably constitutes the receptacle of enzymes which are concerned with syntheses of various food reserves found in different groups of plants.

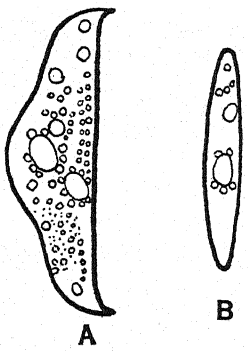


FIG. 1.—Grouping of oil drops around pyrenoids of diatoms: *A*, *Rhopalodia gibba; B*, *Synedra affinis* var. *fasciculata*.

DUFRENOY (6), discussing the physiological significance of the vacuome, states that the vacuoles are storage places for phenolic compounds which act as H carriers in cellular oxidation reduction and that the cytochrome oxidase has been identified in the vacuolar solution, and thus the vacuoles possess the properties of respiratory systems.

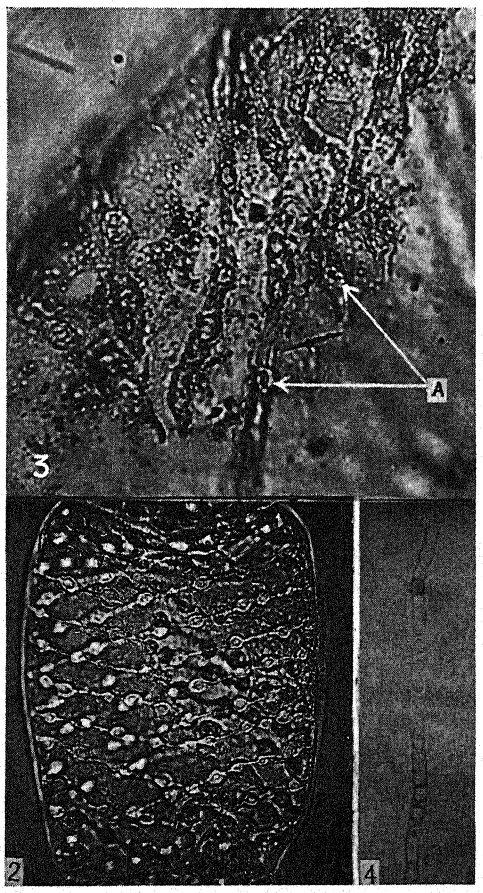
Experiments with algae

Healthy, deep-green filaments of *Spirogyra* in the vegetative stage usually show abundant starch around the pyrenoids in chromatophores (fig. 2) but no oil. PAL (9) reported a similar finding. Oil formation takes place at the conjugating stage by the gradual conversion of starch into oil. Filaments were grown in Erlenmeyer flasks in sterilized oleic acid, glycerin, and bile salt medium (designated hereafter as medium no. 1), some of the flasks being kept in darkness and others in light. Abundant oil was found in the cells of the filaments in the course of 3–5 days, some of the oil drops being actually imbedded around the pyrenoids within the starch sheath in the chromatophores (fig. 3*A*). The oil formation was greater in darkness than in light. Filaments similarly grown in sterilized distilled water as a control showed no trace of oil formation. *Spirogyra* in oleic-acid medium soon turned unhealthy and decomposed. Another fatty acid (caproic) was used in place of the oleic acid to constitute medium no. 2, and the same result was obtained. Media nos. 1 and 2 were made up in the following way: for no. 1, 1 gm. of bile salt was dissolved in 100 cc. of water and 0.5 cc. (= 130 mg.) of oleic acid and 16 drops of glycerin added, the medium being diluted four to six times with distilled water. For medium no. 2, 0.25 cc. (= 115 mg.) of caproic acid was used and the other constituents were of the same amounts. The addition of bile salt helps to make a thin emulsion, so that *Spirogyra* can continue growing for a few days. Both media were sterilized before use in an autoclave at

three atmospheres' pressure for half an hour, and the pH values of the media were about 5.8 and 6. The chemical analysis, kindly carried out by Miss ASHIMA MUKHERJEE under the direction of Dr. P. K. BOSE, confirms the utilization of oleic and caproic acids, as the amounts of both acids decreased during the process, while in two control flasks containing sterilized media nos. 1 and 2 but no filaments, the amounts of oleic and caproic acids remained practically the same.

Subsequently, glacial acetic acid and butyric acid (media nos. 3 and 4, respectively) were used. In each case two varieties (nos. 3*a* and 3*b* and nos. 4*a* and 4*b*) were prepared. In one (3*a* and 4*a*), 100 mg. of the acid was added to 200 cc. of distilled water, with 16 drops of glycerin, and in the other (3*b* and 4*b*), the glycerin was altogether omitted. All these media were sterilized, and their pH values were 5.8 after sterilization. Healthy filaments of *Spirogyra* were plunged into all the media. After 3 days in no. 3*a*, abundant oil formation was noticed in the cells of the filament, the first-formed oil drops being invariably grouped around the pyrenoids within the starch sheath, but the oil formation was much less in no. 3*b*. The same result was repeated with nos. 4*a* and 4*b*. The original starch sheath around the pyrenoids remained intact in all such cases. The chemical analyses confirm the microscopical observation on oil formation; in medium no. 4*a*, 77.5 mg. of butyric acid out of 100 mg. was used, while in the medium no. 4*b*, 67.95 mg. was used.

The estimation of free acids was carried out in each case by titrating the entire medium against the standard N/20 alkali (KOH), with phenolphthalein as an indicator. From these experiments it is concluded that oil is synthesized in the vegetative filament of *Spirogyra* growing in fatty acids and glycerin medium by the secretion of the enzyme lipase, which is probably located in the pinkish vacuoles of the pyrenoids; in the medium lacking in glycerin there was probably some secretion of glycerin from the cells for the synthesis of oil in its filaments. In all cases the first-formed oil drops were deposited around the pyrenoids. The pH value of the medium without glycerin remained the same (5.8), while



FIGS. 2–4.—Fig. 2, starch sheath around pyrenoids of normal, healthy *Spirogyra* cell. Fig. 3, grouping of oil drops (*A*) around pyrenoids within starch sheath in *Spirogyra* cell. Fig. 4, vacuoles near tip of growing hypha of *Polystictus sanguineus*.

that of the medium with glycerin was reduced to 5.5, and the filaments became unhealthy and gradually decomposed. The lowering of pH value is probably due to liberation of other organic acids by the plant during growth. This statement is further supported by the fact, as described below, that after the growth of *Spirogyra* in sterilized 4 per cent glucose medium (with pH 7 at the outset), the pH value decreased to 5.5.

To detect the presence of oil, the filaments were first treated with 0.1 per cent sodium carbonate, or in some cases with 2 per cent caustic potash for half an hour and then washed thoroughly with water to remove the adhering salts and glycerin; they were now tested by Sudan IV as well as by osmic acid. For the osmic-acid test the tannin was removed from the filaments by boiling them in water three or four times, using fresh water each time.

Spirogyra in sterilized 4 per cent glucose medium (with pH 7) in flasks maintains its green color for 8–10 days, during which period it forms abundant starch around the pyrenoids but there is no trace of oil formation. Subsequently it loses the green color and abundant oil formation is noticed around the pyrenoids within the starch sheath, which becomes perceptibly thinner and narrower, gradually decomposing, the pH value of the medium decreasing to 5.5. It has often been found that in vegetative filaments of *Spirogyra* undergoing decomposition in artificial media, as well as in yellowish green (unhealthy) filaments growing in tanks, oil drops are secondarily formed from the primarily formed starch grains situated around the pyrenoids; thus the starch sheath around the pyrenoids always becomes thinner. In all such cases the oil drops are first formed around the pyrenoids within the starch sheath as well as surrounding the sheath, apparently indicating secretion of lipase from the pyrenoids for formation of this oil.

Two species of diatoms, *Rhopalodia gibba* and *Synedra affinis* var. *fasciculata*, were grown in three sterilized media: (*a*) 2 per cent glucose, (*b*) medium no. 1, and (*c*) medium no. 1 but without glycerin. Oil developed in all three, but the amount in *b* was greater than in the other two, and the first deposit of oil in all such cases was around the pyrenoids in the form of small droplets (fig. 1).

Specimens of the Myxophyceae (*Lyngbya* sp. and *Oscillatoria* sp.) which never have any pyrenoids, when grown in 2 per cent glucose medium distinctly show by the Sudan IV test a number of oil globules grouped around the single vacuole in each cell of the trichome.

Experiments with higher plants and fungi

Short green shoots of *Selaginella* sp. were grown in sterilized medium no. 1 and in sterilized medium no. 1 but without glycerin; in both cases the leaves became discolored in 3–4 days and showed copious oil formation, the globules being mostly grouped around the pinkish vacuoles in the plastids. In 4 per cent glucose medium, oil formation took place a little later, in about 6–8 days. Normal green leaves of *Selaginella* do not contain oil; they show chloroplastids with central pinkish vacuoles and starch is usually deposited around such plastids. In green leaves of higher plants also such pinkish vacuoles inside the plastids are surrounded by a thin layer of starch.

By growing a number of fungi, *Mucor* sp., *Chaeotomium* sp., in sterilized medium no. 1 and sterilized medium no. 1 but without glycerin, it was found that

oil drops were more numerous in the medium with the glycerin. Similarly, *Chaeotomium* sp., *Aspergillus oryzae*, *Polystictus sanguineus*, and *Polyporus agariceus* grown in 4 per cent glucose medium increased the amount of glycogen considerably. The chemical test by hydrolysis with hydrochloric acid and treatment with Fehling's solution confirmed the increase of glycogen. These are clear instances of synthesis of fat and glycogen by means of appropriate enzymes, which are probably located in the pinkish vacuoles of actively growing hyphae (fig. 4).

Discussion

The pinkish vacuolar stain seems very stable. It does not dissolve in alcohol, acetone, petrol-ether, ether, xylol, chloroform, iodine solution, benzol, lactic acid, acetic acid, hydrogen peroxide, pyridine, caustic potash (2 per cent) etc., although the vacuole shrinks in many cases. With Wratten filters the faint color remains visible and also under the dark-field illumination. Treated with 1 per cent antimony trichloride in chloroform solution, a slightly bluish tinge is produced in such vacuoles, surrounded by a reddish tinge, which, however, is not soluble in ether or petrol-ether, indicating that the stain is not of the nature of carotenes. Probably such vacuoles—being receptacles of enzymes—are protected by a plasma membrane (10). According to RANJAN, "the immunity of enzymes in living cell from the attacks of other enzymes may also be due to the membranes surrounding them. If we agree with WILLSTÄTTER that enzymes in general are adsorbed on a large colloidal carrier, forming a 'symplex,' then at the interface a membrane may be deposited, which will then act as a protective cover. Such types of enzymes will need the rupture of the cell to liberate them."

Structurally the pyrenoid consists of a central core of proteinaceous nature. According to recent views, the pyrenoid is not crystalloidal in character. CZURDA (5) states that the pyrenoid is a viscous mass of protein and that its interior is invariably structureless. The central portion of the pyrenoids gives the protein test with Millon's reagent. NORTHROP (8) has discussed crystalline enzymes in detail.

In plants with pyrenoids, syntheses of food reserves are usually found around the pinkish vacuoles of the pyrenoids. KARSTEN (7) states that the actual formation of oils in connection with pyrenoids is rare in starch-free diatoms. In *Rhopalodia gibba* and *Synedra affinis* var. *fasciculata*, however, the distinct grouping of all oil drops around the pyrenoids was observed (fig. 1). In plants lacking in pyrenoids, pinkish vacuoles are always found inside the plastids, and food substances are usually deposited in connection with them. In the case of fungi, which have always pinkish vacuoles at their tips (fig. 4), fat and glycogen contents can be increased by growing them in fatty acid and glucose media, respectively. The present observations and experiments indicate that vacuoles are really the receptacles of enzymes for the construction of various food substances, whether such vacuoles are located inside the pyrenoids, or plastids, or fungal hyphae.

Summary

1. In members of the Chlorophyceae with pyrenoids, starch is usually synthesized around the pyrenoids.

2. In starch-free diatoms the first-formed oil drops are grouped around the pyrenoids.

3. Healthy green filaments of *Spirogyra* in the vegetative stage usually show accumulation of starch but no oil. But by growing *Spirogyra* in fatty acids and glycerin medium, oil can be synthesized in the vegetative filaments; here also the first-formed oil drops are found around the pyrenoids within the starch sheath. Chemical analyses confirm the utilization of acids in the process.

4. In vegetative filaments of *Spirogyra* undergoing decomposition in artificial media, as well as in yellowish green (unhealthy) filaments growing in tanks, oil drops are secondarily formed from the primarily formed starch grains situated around the pyrenoids. In all such cases the oil drops are first formed around the pyrenoids within the starch sheath as well as surrounding the sheath. There must therefore have been secretion of lipase from the pyrenoids for formation of this oil.

5. A faint pinkish stain is visible under the oil immersion lens in the pyrenoids at their center. In plants lacking in pyrenoids, pinkish vacuoles are always found inside the plastids, and food substances are usually deposited in connection with them. Such vacuoles are found in growing hyphae of diverse groups of fungi, being evidently connected with their general metabolism. In many fungi, fat and glycogen contents can be increased by growing them in fatty acid and glucose media, respectively.

6. The pinkish vacuoles are considered receptacles of enzymes for syntheses of various food reserves in plants, lower as well as higher.

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LITERATURE CITED

1. BOSE, S. R., Pinkish vacuolar stain in growing tips of fungal hyphae in artificial culture. Curr. Sci. 8:170–171. 1939.
2. ———, The nature of the colouring substances of some coloured Polyporaceae. Trans. Nat. Inst. Sci. India 2:69–85. 1941.
3. ———, Function of pyrenoids in algae. Nature 148:440–441. 1941.
4. CLELAND, R. E., The cytology and life-history of *Nemalion multifidum* Ag. Ann. Bot. 33:323–351. 1919.
5. CZURDA, V. H., Morphologie und Physiologie des Algen-stärkekornes. Beih. Bot. Centralbl. 45:97–270. 1928.
6. DUFRENOY, J., On the physiological significance of the vacuome. Biodynamica 3:171–190. 1940.
7. KARSTEN, G., ENGLER'S Naturliche Pflanzenfamilien. Leipzig. 1928.
8. NORTHROP, J. H., Monograph on crystalline enzymes. Columbia Biol. Ser. No. 12. 1939.
9. PAL, N. L., Studies on the respiration of conjugating *Spirogyra* with special reference to fat metabolism. New Phytol. 33:241–273. 1934.
10. RANJAN, S., Membranes and surface reactions. Proc. 27th Ind. Sci. Congress, part IV. 1940.
11. SMITH, G. M., Fresh water algae of the United States. McGraw Hill, New York. 1933.
12. ———, Cryptogamic botany. Vol. I. McGraw Hill, New York. 1938.
13. ———, *Ibid.*, vol. II. 1938.
14. STEINECKE, F., and ZIGENSPECK, H., Über Pyrenoidveränderungen bei der Stärkebildung in *Spirogyra*-Zellen. Ber. deutsch. Bot. Ges. 46: 678–681. 1928.
15. ZIRKLE, C., The structure of the chloroplast in certain higher plants. Parts I, II. Amer. Jour. Bot. 13:300–320; 321–341. 1926.

EFFECTS OF GROWTH-REGULATING SUBSTANCES ON A PARTHENOCARPIC FRUIT[1]

HAROLD E. CLARK AND KENNETH R. KERNS

Introduction

In the course of experiments on the effect of several synthetic growth-regulating substances on development of the fruit of the pineapple, *Ananas comosus* (L.) Merr., a definite stimulation of the growth of the fruit has been noted following the application of relatively high concentrations of certain of them.

It has already been reported (1) that low concentrations of α-naphthaleneacetic acid applied prior to normal floral differentiation induced premature flowering of the pineapple, but high concentrations applied at the same time delayed flowering. The following data were obtained from plants treated with naphthaleneacetic acid after completion of normal floral differentiation but prior to actual blossoming (8). Some reference will also be made to results from plants treated with Fruitone, a proprietary material which includes synthetic growth regulators similar to naphthaleneacetic acid in their effects.

Experimental methods

The plants used in the experiments were of the Cayenne variety, grown in the field in the customary manner (10) in a two-row bed from a fall planting of slips. Normal floral differentiation began about 14 months after planting, as determined by sectioning and examining a few of the plants. In a uniform fall planting of this type, most of the plants start floral differentiation within an interval of about 1 week (8). From observations made in the same locality (8) of the time required for completion of the process of floral differentiation, it can be assumed that in this test the primordia of all flowers of the inflorescence had been formed within 6 weeks after the first stages—visible under the microscope—of transition from a vegetative to a floral meristem.

The experimental variables were initiated approximately 2 weeks after primordia of all flowers had been formed. At the time the treatments were applied, the young inflorescence (less than 1 inch in diameter) was still partly surrounded by leaves in the center of the plant, not having reached the stage of actual blossoming, which began about 1 month later. The plants had received adequate fertilization previously and were of average size (about 12 pounds) and quite uniform in quality of growth. Each plot included forty plants. Solutions of α-naphthaleneacetic acid (table 1) were sprayed on the surface of the leaves and inflorescence. The chemical was first dissolved in a small amount of alcohol, which was then diluted with water, and 1 liter was applied to each plot. The controls remained untreated.

Fruits were picked when ripe, each fruit being weighed to the nearest ounce. At the same time the number of fruitlets along the longest ascending spiral of the fruit (fig. 1) was counted. After the fruits had been harvested, the number of developing slips and suckers on each of the plants was recorded.

The significance of the effect of the treatments on weight of fruit and number of fruitlets was determined by analysis of variance (11). The significance of the difference between treated and control plots in numbers of slips per plant was determined by separating the plants into two categories: those with two slips

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or more and those with less than two. These data were submitted to the chi-square test (11). Records of numbers of suckers were segregated and tested in the same manner.

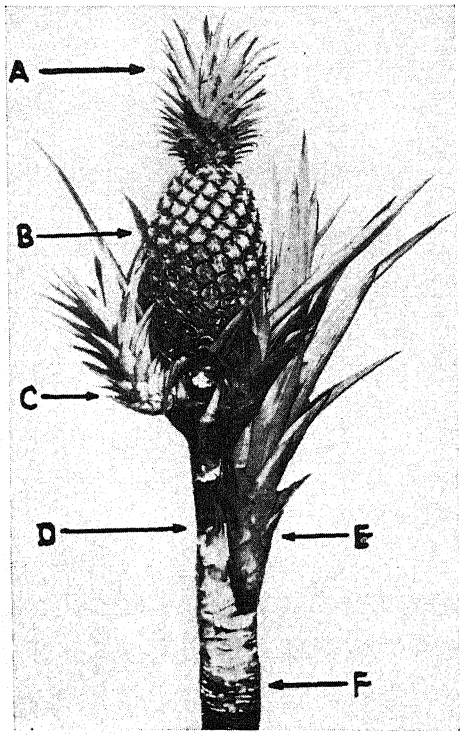


FIG. 1.—Pineapple plant at ripe-fruit stage with leaves removed from stem to show *A*, crown; *B*, fruit; *C*, slip; *D*, peduncle; *E*, sucker; and *F*, stem.

Experimental results

FRUITS

Higher concentrations of naphthaleneacetic acid definitely increased the weight of the fruits (table 1), but the lower concentrations had no significant effect in comparison with the controls. According to the calculated F values in table 1, the number of fruitlets per spiral had not been affected significantly by the treatments. This result was anticipated, since all the fruitlets presumably had been initiated and differentiation of the leaves of the crown at the apex of the fruit had been resumed when the naphthaleneacetic acid was applied. Other plots which received similar treatments considerably later (during the period of actual blossoming) gave similar results, with no real influence of treatments on the number of fruitlets but with marked increase in the size of the fruit following application of high concentrations of the acid. The highest concentration delayed ripening a week or more (fig. 2), although none of the other treatments influenced the time of ripening. A greater retardation, approximately 1 month, was obtained in other plots by application of the same high concentration prior to normal floral differentiation.

TABLE 1

INFLUENCE OF CONCENTRATION OF NAPHTHALENEACETIC ACID ON WEIGHT OF FRUIT AND NUMBER OF FRUITLETS PER SPIRAL WHEN APPLIED AFTER NORMAL FLORAL DIFFERENTIATION

	Concentration of acid (%)	Average fruit weight (lb.)	Fruitlets per spiral no.
	0.001	5.08	18.2
	.003	5.26	19.0
	.01	5.47	18.6
	0.05	5.98	18.5
	Controls	5.27	18.7
F values	Obtained	10.02	1.46
	Required, p=0.05	2.42	2.42
	Required, p=0.01	3.41	3.41
Difference necessary for significance	p=0.05	0.31	.....
	p=0.01	0.41	.....

Further measurements of the fruits in plots referred to in table 1 were not taken, but comparable data showing some effects of a high concentration of the growth regulators on size and quality of fruit were obtained in adjacent plots.

For example, a plot treated with Fruitone at the same time as those given in table 1 can be compared with a group of four control plots. The Fruitone used was obtained in 1941, but its exact composition is not known. Other experiments with pure naphthaleneacetic acid and naphthylacetamide have shown that either stimulates growth of the pineapple fruit. The Fruitone plot received a solution calculated to provide the same amount of effective growth substance that was applied to the naphthaleneacetic-acid plot

TABLE 2

INFLUENCE OF FRUITONE ON WEIGHT AND DIMENSIONS OF FRUIT AND ON NUMBER OF FRUITLETS PER SPIRAL WHEN APPLIED AFTER NORMAL FLORAL DIFFERENTIATION

Treatment	Average fruit weight (lb.)	Fruitlets per spiral	Fruit dimensions	
			Length (cm.)	Diameter (cm.)
Fruitone....	6.54	18.7	22.4	15.3
Controls....	5.21	18.7	21.0	14.2

treated with 0.05 per cent solution. In this Fruitone plot, however, the solution was poured into the center of the plant directly on the inflorescence instead of being sprayed on the leaves. Data from Fruitone and control plots were obtained from twenty representative fruits selected from each plot (table 2).

It is apparent that the heavier, treated fruits were slightly larger in all dimensions, although the number of fruitlets was the same as in the controls, the individual fruitlets being noticeably larger in the treated fruits than in the untreated.

Within the flesh of the fruit there was no apparent anatomical effect from the growth substances. Both treated and control fruits were translucent and nonporous, that is, the tissues were well filled with juice. It should be mentioned, however, that the concentration of the juice of the treated fruits was about 2° Brix[2] lower than that of the controls (14.0° and 16.0°, respectively).

PEDUNCLES

Development of the peduncle was modified considerably by the treatments

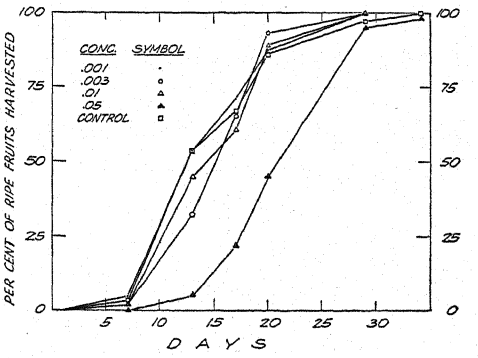


FIG. 2.—Cumulative harvest of ripe fruits from various plots according to date of ripening to show interval in days covered by harvest.

which resulted in the greatest increase in weight of fruit. Anatomical studies have not yet been completed, but in the plots receiving the highest concentration of naphthaleneacetic acid the peduncles were longer and slightly thicker than those of the controls. Furthermore, some difficulty was experienced in separating the fruits from the peduncles in these plots at the time of picking, in contrast with the ease of separation in the untreated plots or in those receiving a low concentration of the acid.

SLIPS AND SUCKERS

The treatment also had some effect on the development of axillary buds, particularly those which develop into the slips

[2] Brix hydrometer, with scale readings of percentage sugar by weight, gives some indication of sugar content of a plant sap in which sugar comprises most of the soluble solids.

(fig. 1), that is, the branches in the axils of the leaves on the peduncle. Rudimentary slip buds are present by the time the inflorescence has been formed, but most of these remain dormant and only a few develop further into axillary branches. The data in table 3 show a tendency for increase in the number of slips which developed with the lower concentrations, the result being highly significant only in the plot receiving the 0.003 per cent solution. This tendency was also noted in other plots treated somewhat later than those referred to in table 3. In contrast, plots receiving a high concentration in the center of the plant showed considerable restriction of slip development. For example, the Fruitone plot had only 0.36 slips per plant as against the 1.45 in the controls.

There was no significant influence of the treatments referred to in table 3 on the development of suckers—the axillary branches on the stem of the plant (fig. 1). In other plots high concentrations applied in the center of the plant caused some restriction in the development of the suckers, although not so marked as the inhibition of the slips in the same plots.

TABLE 3

INFLUENCE OF CONCENTRATION OF NAPHTHALENEACETIC ACID ON GROWTH OF SLIPS AND SUCKERS WHEN APPLIED AFTER NORMAL FLORAL DIFFERENTIATION

Concentration of acid (%)	Slips				Suckers			
	Average no. per plant	No. of plants with		Probability of homogeneity with controls (%)*	Average no. per plant	No. of plants with		Probability of homogeneity with controls (%)*
		One or less	Two or more			One or less	Two or more	
0.001	2.30	18	22	15	1.90	8	32	40
0.003	2.40	13	27	< 1†	2.08	7	33	25
0.01	1.35	26	14	80	1.85	10	30	80
0.05	1.42	24	16	80	1.68	17	23	15
Controls	1.45	25	15	.....	1.75	11	29	.....

* Based on chi-square test. † Significantly different from controls.

Discussion

Aitionomic parthenocarpic fruits have been produced by synthetic growth substances (4), including naphthaleneacetic acid (2) and naphthoxyacetic acid (9, 13). Indolebutyric acid has been used with tomatoes to increase the set of blossoms (6, 7), and some increase in size of fruit above that of the controls was obtained during the first few weeks of harvesting. Seedless tomato fruits produced by treatment with naphthoxyacetic acid were also reported (5) to be slightly larger than the controls. Although the pineapple fruit is autonomically parthenocarpic, the further stimulation of its growth by growth substances may be the result of processes similar to those occurring in fruits which are aitionomically parthenocarpic.

Although the pineapples in the plot receiving the highest concentration of naphthaleneacetic acid ripened a week later than the controls, the larger size of these treated fruits could not have been

due to a longer period of growth, because their greater size was apparent before they attained ripeness.

The pineapple fruit does not become detached from the peduncle without external force, but the difficulty in separating the fruit from the peduncle in the treated plots probably is significant in relation to known effects of naphthaleneacetic acid and naphthylacetamide upon abscission. For example, these substances have been reported to delay abscission of apples from their peduncles (3), although the concentration used was far less than that which had the greatest effect on the peduncles and fruit of pineapples.

The effect of the growth substances on the peduncles probably was not a dominant factor affecting the size of the fruit. Instead, a direct stimulation of the growth or expansion of the fruit itself by the action of the naphthaleneacetic acid was indicated.

It has been pointed out (12) that the concentration of auxin required for maximum growth varies with different organs and that higher concentrations inhibit growth. With pineapples, a relatively high concentration of naphthaleneacetic acid was apparently necessary for stimulation of growth of the fruit, and the concentration that inhibited enlargement of fruit was not reached in this experiment. A much lower concentration stimulated dormant slip buds into growth, but a high concentration inhibited the development of many slip buds.

Summary

1. Application of sprays of α-naphthaleneacetic acid to pineapple plants after normal differentiation of floral primordia had been completed influenced the growth of fruit, peduncle, and axillary branches.

2. Relatively high concentrations of the growth-regulating substances caused a marked increase in size and weight of ripe fruit, although the number of fruitlets was not influenced by any of the treatments. This increase in growth of the fruit was accompanied by the development of large peduncles, from which the fruit was separated with some difficulty.

3. The highest concentration of naphthaleneacetic acid also delayed ripening, and, particularly when applied in the center of the plant, partially inhibited growth of slips and suckers.

4. Relatively low concentrations applied at the same time caused some stimulation of the growth of dormant slip buds, resulting in a larger number of slips per plant.

The writers express their appreciation to Mr. C. A. FARDEN for assistance in statistical analysis of the data. A stimulation of their interest in growth regulators was an experiment to delay flowering by application of such substances prior to normal floral differentiation initiated by Dr. FOLKE SKOOG when at the Pineapple Research Institute.

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LITERATURE CITED

1. CLARK, H. E., and KERNS, K. R., Control of flowering with phytohormones. Science **95**:536–537. 1942.
2. GARDNER, F. E., and MARTH, P. C., Parthenocarpic fruits induced by spraying with growth-promoting compounds. BOT. GAZ. **99**:184–195. 1937.
3. GARDNER, F. E., MARTH, P. C., and BATJER, L. P., Spraying with plant growth substances to prevent apple fruit dropping. Science **90**:208–209. 1939.
4. GUSTAFSON, F. G., Inducement of fruit development by growth-promoting chemicals. Proc. Nat. Acad. Sci. **22**:628–636. 1936.

5. ———, β-naphthoxyacetic acid as an inductor of parthenocarpy in tomatoes. Proc. Amer. Soc. Hort. Sci. 40:387–389. 1942.
6. HOWLETT, F. S., Fruit set and development from pollinated tomato flowers treated with indolebutyric acid. Proc. Amer. Soc. Hort. Sci. 41: 277–281. 1941.
7. ———, Effect of indolebutyric acid upon tomato fruit set and development. Proc. Amer. Soc. Hort. Sci. 39:217–227. 1941.
8. KERNS, K. R., COLLINS, J. L., and KIM, HAROLD, Developmental studies of the pineapple, *Ananas comosus* (L.) Merr. New Phytol. 35: 305–317. 1936.
9 MITCHELL, J. W., and WHITEHEAD, MURIEL R., Effects of vaporous naphthoxyacetic acid on development of tomato fruits, with special reference to their vitamin C content. BOT. GAZ. 104:362–365. 1942.
10. NIGHTINGALE, G. T., Nitrate and carbohydrate reserves in relation to nitrogen nutrition of pineapple. BOT. GAZ. 103:409–456. 1942.
11. SNEDECOR; G. W., Statistical methods. 3d ed. Iowa State College Press. 1940.
12. THIMANN, K. V., Hormones and the analysis of growth. Plant Physiol. 13:437–449. 1938.
13. ZIMMERMAN, P. W., Growth regulators of plants and formative effects induced with β-naphthoxy compounds. Proc. Nat. Acad. Sci. 27:381–388. 1941.

NUCLEOLAR STAIN AND NUCLEAL REACTION

C. S. SEMMENS

The technique introduced by SEMMENS and BHADURI (19) actually involves only three simple steps: (*a*) treatment with an alkali carbonate mordant; (*b*) staining with a dye solution; (*c*) washing out unattached dye. The method, used in conjunction with the nucleal reaction of FEULGEN and ROSSENBECK (9), as it is intended to be, involves the chemical differentiation of nucleolar material from chromatin; therefore certain essential conditions of the material, depending on previous treatments such as fixation, must be attended to if the process is to yield the required results. The nucleolar stain is designed to follow and to contrast with the Feulgen staining of chromatin; the hydrolysis necessary for the clear chromatin stain also brings about an essential change in the nucleolar material and is therefore equally necessary for the successful staining of the nucleolus by this method.

In the crystal-violet iodine technique as used for mitotic and meiotic material, the nucleolus is more or less deeply stained, and any association between chromosomes and nucleoli is difficult and usually almost impossible to determine with certainty. This is also the case with the iron-alum haematoxylin method. With the Feulgen technique the nucleoli are not stained at all, in spite of the close chemical relationship between crystal violet and Fuchsin. Here the chromosome-nucleolus relationship is again impossible to determine accurately. The Gram technique as applied to the staining of chromosomes and nucleoli does not provide a specific chromatin stain. LUDFORD (16) obtained a certain measure of success with a green dye used as a counterstain after Feulgen staining. Of course, an ordinary simple counterstain cannot serve the same purpose as a specific stain; the optical effect of dispersed green dye renders minute details of satellite or filament obscure, and there is always the physical possibility of optical resolution being hindered by dye adsorbed between filament and nucleolus. Failure to distinguish between adsorbed and attached dye has led to some rather extraordinary claims.

HILLARY'S recent use of simple counterstaining with green dye (13) has added little to the work of earlier investigators. The simple chemical difference between light green and fast green he does not mention. Moreover, the possibility that light green may form a stable or fadeless compound by actual combination with certain types of material (as it does with the nucleolus in my experience) is seldom taken into account.

Whether the nucleolar staining method of SEMMENS and BHADURI (3, 4) actually satisfies the technician's demand for a definite and specific stain for nucleolin, to go with the only definite and specific stain for chromatin (technique of FEULGEN and ROSSENBECK), is a matter for the test of time and expert consideration. Verbatim repetition of unsound criticism (7) is not authoritative opinion.

The original paper of SEMMENS and BHADURI (19) concerned a method of differentiating nucleoli from chromosomes. The reference to the advisability of avoiding acetic acid in the fixative had therefore nothing to do with the chromatin staining, although HILLARY (12) seems to think that it had. The Feulgen technique is an established method; the paper referred to by HILLARY was concerned with the nucleolus. The wording in that paper was not so obscure as to make it appear that the caution about acetic acid referred to a possible effect on the Feulgen staining, especially as this

action of acetic acid had already been indicated by the work of earlier investigators (16, 1, etc.).

Acetic acid was found to have a definite action on the nucleolus, as a result of which the nucleolin, after acid hydrolysis and subsequent treatment with alkali carbonate, would not retain the green dye if the material were subjected to adequate washing. The change that actually takes place in the constitution of nucleolin as a result of the action of acetic acid has a chemical significance which will be discussed in another article. HILLARY'S reference to the action of acetic acid relates to the chromatin staining by the Feulgen technique. The remarks about acetic acid in the paper on nucleolar staining by SEMMENS and BHADURI concern the nucleolar staining and should not be disregarded. To stain nucleoli specifically by this method, acetic acid should be avoided in the fixative and chromic acid used, either in the fixative itself or as a subsequent mordanting bath. Although the technique is extremely simple, the essential chemical conditions for the final reaction are necessarily slightly involved. One is the action of metabisulphate as a nucleolar mordant for acid green dyes which has been previously referred to by SEMMENS (17), and HILLARY'S (13) staining appears to be a good sample of this action. In my experience, however, the method is not applicable: in many cases the stain is fugitive, and it is much less distinctive than the effect after separate carbonate mordanting.

JOHANSEN (15) has stated that the Fuchsin stain in the chromosomes is "often completely removed," presumably by the alcoholic carbonate used as a mordant for the nucleolus. This difficulty has never arisen in our laboratory, and it is hard to understand from the chemical point of view why it should occur if the technique is strictly followed. It has been assumed (17–20) that the effect of alkalies on Fuchsin staining would be generally known, and no warning was therefore thought necessary about the action of the mordant.

In the mordant solution, and in the alcohols used for washing out surplus mordant, preparations normally appear almost colorless and may probably have been regarded as spoilt, since little chromatin stain is apparent under the microscope. The usual reddish magenta color of the chromatin reappears, however, in the subsequent treatment of the material, if the correct period of hydrolysis has been given (30 minutes at 60° C. for chromic-fixed plant material, 20 minutes at 40° C. for animal material). Preparations should not be discarded simply because they appear colorless in alkaline solution. The Fuchsin dye is chemically linked to the chromatin by the Feulgen and Rossenberg technique, and although internal rearrangement of linkages in the complex molecule thus formed may take place under the influence of mild alkali, leading to a temporary leuco condition, the chromogen is readily re-established when the alkali is completely washed out in the rinsing alcohols and the prevailing pH sufficiently lowered by the subsequent treatments. In the experience of a number of cytologists the final appearance of the chromatin is rather more brilliant after the nucleolar staining than with the simple Feulgen technique alone, the chromatin specificity of the Feulgen stain being in no way impaired. It is possible that loss of color after the mordant is because the solutions for subsequent treatment were somewhat on the alkaline side, and those who have difficulty possibly adhere to the time and tempera-

ture of hydrolysis—4 minutes at 60° C.—tentatively suggested by FEULGEN in the original paper.

Light green was chosen as a stain for the nucleolus in the nucleolar staining method (19) on account of its acid nature and its value as a contrast to magenta. As pointed out by SEMMENS (17, 18), fast green works equally well but gives a bluish tinge, which, though it does not appear to be satisfactory to the writer, may have definite advantage for those whose sensitivity to green is less keen than to blue. A number of other dyes of similar chemical constitution to light green will also give equally good results if applied according to the technique.

The dye solution itself is a critical factor in the technique, and if attempts to stain the nucleolus specifically with ordinary light green solutions lead to failure, such failure must not be credited to the S. & B. nucleolar technique. A trace of aniline in the solution is essential to the dye linkage, but the presence of much nitrobenzene (the most usual impurity with aniline oil) is distinctly inimical and will lead to disappointing results. If the method, as recently published (2, 20), is carefully adhered to, good preparations can be obtained with little trouble. As with all new techniques, however, patience is necessary at first.

The nucleolar staining method actually introduces only a single stage in addition to the ordinary procedure for double staining with Feulgen technique and a counterstain, as used by LUDFORD (16), HILLARY (13), and others. This additional stage, however, is a very important one—the application of a mordant for a specific secondary staining purpose subsequent to the attachment of a separate dye in a primary locus. It is the use of a mordant for the purpose of introducing a second stain specific for a given subsidiary component after the application of a primary stain specific for a major component that constitutes the new feature. This mordant treatment makes all the difference between a generally dispersed counterstain, with its characteristic optical effects, and a definitely located or specific stain.

In previous publications dealing with the nucleolar technique the terms "mordant" and "differentiating solution" have been used for convenient description. The main features of the nucleolar staining method are here compared with those of the nucleal reaction:

Nucleal reaction	Nucleolin reaction
Certain preliminary restrictions on nature of fixatives.	Certain preliminary restrictions on nature of fixatives.
New treatment for material; acid hydrolysis at 60° C. in N/HCl after fixation.	New treatment of fixed material; mild alkali hydrolysis subsequent to acid hydrolysis and treatment with sulphite solution.
Special dye solution for specific staining.	Special dye solution for specific staining.
Unattached dye removed with special acid solution.	Unattached dye removed with special alkali solution.
Time of hydrolysis and temperature tentative and subject to variation to suit material.	Strength of mordant and length of immersion tentative and subject to adaptation to suit material.

Consideration of the elementary chemistry of dye constitution will make it apparent that:

Nucleal reaction	Nucleolin reaction
The substitution of crystal violet (18), malachite green, pararosaniline, methyl green, etc., for basic Fuchsin could not be regarded as constituting new techniques.	The use of fast green, Guinea green, aniline blue, etc., or similar acid dyes in place of light green or mordant treatment after dye (instead of before it) could not be considered as new techniques.

SHEFFIELD (21), employing the S. & B. technique for comparative purposes in connection with very exacting cytochemical investigations on plant viruses, reports favorably on its use. CASPERSSON and SCHULTZ (5) have suggested a chemical explanation for the action of the mordant and for the reaction between dye and nucleoli. The possibility of adequate mordanting, for the green dye to be retained by the nucleoli of certain materials, simply by treatment with potassium metabisulphite solution after staining in Fuchsin was pointed out some time ago (17) and has been borne out more recently by HILLARY (13) and DELAY (8).

If the material is not adequately washed in the so-called differentiating solution after treatment with green dye, the dye will adhere rather tenaciously in such loci as lend themselves to adsorption (for example, the surface of chromosomes and certain small characteristic cytoplasmic inclusions). Such adsorbed dye may lead to questionable interpretations (14); thorough and careful washing, however, should remove all dye held simply in physical relation.

BHADURI (2) has provided evidence of the value of the combined nucleal and nucleolin techniques, as well as of the inadequacy of many of the earlier staining methods in the matter of differentiating and rendering visible the finer details of nuclear organization. Already the volume of literature on the chemical aspects of cytology is reaching considerable proportions, and a number of cytologists (6, 10, 11, 22, and others), some of whom formerly concerned themselves mainly with the morphological and mechanical aspects of chromosome behavior, are now showing awareness of the significance of this modern trend.

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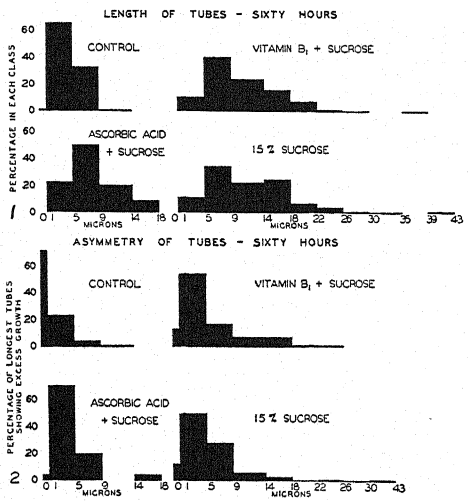
LITERATURE CITED

1. BAUER, H., Die Feulgensche Nuklealfärbung in ihrer Anwendung auf cytologische Untersuchungen. Zeit. Zellf. Mikr. Anat. 15:225–247. 1932.
2. BHADURI, P. N., Cytological analysis of structural hybridity in *Rhoeo discolor* Hance. Jour. Genet. 44:73–85. 1942.
3. ———, Application of new technique to cytogenetical reinvestigation of the genus *Tradescantia*. Jour. Genet. 44:86–127. 1942.
4. BHADURI, P. N., and SEMMENS, C. S., Nucleolar staining method applied to animal tissues. Jour. Roy. Mic. Soc. 62:21–24. 1942.
5. CASPERSSON, T., and SCHULTZ, J., Ribonucleic acids in both the nucleus and cytoplasm and the function of the nucleolus. Proc. Nat. Acad. Sci. 26:507–515. 1940.
6. DARLINGTON, C. D., Chromosome chemistry and gene action. Nature 149:66–69. 1942.
7. DARLINGTON, C. D., and LA COUR, L. F., The handling of chromosomes. Allen and Unwin, London. 1942.
8. DELAY, C., Recherches sur le noyau des Légumineuses. Rev. Cytol. Cytophysiol. Vég. 4:183–230. 1940.

9. FEULGEN, R., and ROSSENBECK, H., Mikroskopischchemischer Nachweis einer Nucleinsäure. Zeitsch. Physiol. Chem. 135:203–248. 1924.
10. GATES, R. R., Nucleoli and related nuclear structures. Bot. Rev. 8:337–409. 1942.
11. GULICK, A., The chemistry of the chromosomes. Bot. Rev. 7:433–457. 1941.
12. HILLARY, B. B., Use of the Feulgen reaction in cytology. I. Effect of fixatives on the reaction. BOT. GAZ. 101:276–300. 1939.
13. ———, II. New techniques and special applications. BOT. GAZ. 102:225–235. 1940.
14. JACOB, K. T., Chromosome numbers and the relationship between satellites and nucleoli in *Cassia* and certain other Leguminosae. Ann. Bot. 4:201–226. 1942.
15. JOHANSEN, D. A., Plant microtechnique. McGraw-Hill, London. 1940.
16. LUDFORD, R. J., Studies in the microchemistry of the cell. I. The chromatin content of normal and malignant cells, as demonstrated by Feulgen's "nucleal reaction." Proc. Roy. Soc. B. 102:397–406. 1928.
17. SEMMENS, C. S., Methods in plant cytology. VI. Fuchsin and light green. Micros. entomol. Monthly 3:177–181. 1939.
18. Improved cytological methods with crystal violet. Stain Technol. 17:147–148. 1942.
19. SEMMENS, C. S., and BHADURI, P. N., A technic for differential staining of nucleoli and chromosomes. Stain Technol. 14:1–6. 1939.
20. ———, and ———, Staining the nucleolus. Stain. Technol. 16:119–120. 1941.
21. SHEFFIELD, F. M. L., The cytoplasmic and nuclear inclusions associated with severe etch virus. Jour. Roy. Mic. Soc. 61:30–45. 1941.
22. WHITE, M. J. D., Nucleus, chromosomes and genes. Part V. Cytology and cell physiology. Clarendon Press, Oxford. 1942.

Attempts to germinate the pollen of the common dandelion are of interest, since there appears to be no record of successful germination of composite pollen *in vitro* (3), and since natural germination has not been recorded (2). This species is apogamous, the embryo developing from a diploid egg; the typical pollen is likewise diploid, but variations ranging from normal reduction to formation of supernumerary grains by chromatin extrusion have been observed (1).

Taraxacum officinale Weber., or *T. vulgare* Lam. Schrk., was tested for germination under a



FIGS. 1, 2.—Fig. 1, percentage of tubes of various lengths after 60 hours under different nutrients. Fig. 2, excess growth of longest tubes in each lot after 60 hours, shown in percentages.

variety of conditions. Ordinary hanging-drop preparations of the usual media failed to give results, but definite, intact, tubular extrusion through all three germ pores was obtained when the pollen was floated on 15 per cent sucrose with coverglass arranged to admit air. The preparations were kept in a small moist chamber at 24°–26°C. As compared with the distilled-water control, in which the tubular extrusions ranged 0–13 μ in length (fig. 1), those of the pollen treated with 15 per cent sucrose were 0–42 μ. The addition of growth-promoting substances (thiamin, ascorbic acid, 3-indoleacetic acid, 3-indolebutyric acid, and 3-indolepropionic acid) had no effect on the maximum length attained. However, the addition of thiamin to the sucrose increased the number of grains showing tubes from 26 to 67 per cent. Addition of ascorbic acid decreased "germination" from 26 to 22 per cent. Other growth-promoting substances, such as takadiastase, yeast extract, and naphthaleneacetic acid, when added to the sucrose produced no tubes larger than those in distilled water. Effects resembling those of ascorbic acid were produced by 3-indoleacetic acid and also by 3-indolebutyric and 3-indolepropionic, but to a less marked degree. The addition of boron had no effect on the action of sucrose.

These tests indicated the tendency of one tube to surpass the others in length. In distilled water only 25 per cent of the tubes surpassed either of the other two by as much as 1–4 μ, and none beyond 5 μ (fig. 2). However, 15 per cent sucrose, both alone and in combination with thiamin or ascorbic acid, had marked effect in producing a single tube or extrusion longer than the two remaining.

The graphs represent conditions after the lapse of 60 hours. In general, the great majority of grains reacting were 30–34 μ in diameter, and presumably diploids formed in dyads. There was no fixed relation between pollen size and magnitude of response, as measured by length of tubes or ascendency of one over the others in length. If tube lengths more than twice the pollen diameter or tubes showing nuclei (3) are accepted as criteria of germination, it cannot be maintained that true germination was secured. The operation of simple osmotic or colloidal swelling is not definitely excluded as an explanation. These seem unlikely, however, in view of the rather frequent predominance of a single tube, the concentration of nutrient used, and the failure to obtain similar phenomena with variations of pH. The rather frequent predominance in length of a single tube, its intact character, and the definite effect of certain nutrient and growth-promoting materials all suggest that true growth was initiated.—ELAINE J. KATZ, *Oberlin College, Oberlin, Ohio.*

LITERATURE CITED

1. SEARS, P. B., Amiotic parthenogenesis in *Taraxacum vulgare* (Lam.) Schrk. and *Taraxacum laevigatum* (Willd.) D.C. Ohio Jour. Sci. 17: 97–100. 1917.
2. STORK, H. E., Studies in the genus *Taraxacum*. Bull. Torr. Bot. Club 47: 199–210. 1920.
3. WODEHOUSE, R. P., Personal communication. 1942.

CURRENT LITERATURE

Practical Plant Anatomy. By ADRIANCE S. FOSTER. New York: D. Van Nostrand Co., 1942. Pp. 155. $2.50.

This compact volume, indicative of present interest and development in the field of plant anatomy, is more nearly a scholarly manual than a comprehensive treatise. The author, recognizing the number and merit of extensive textbooks covering this area, presents an instruction program in more concise form. He aims with a minimum of discussion and direction to guide the student, both in his reading and in his laboratory study. The essence of theory, with copious references, is combined with the study of living and macerated material as well as prepared slides.

For each topic an introduction provides necessary descriptions and definitions, as well as critical discussion of facts, theories, and interpretations, against a classic background. The laboratory directions which follow are somewhat elastic and are applicable either to a general course or to more extended study in plant anatomy. The use of diagrams and notes is recommended in partial substitution for the necessarily tedious drawings of complex structures or tissues. Use of living material is encouraged where possible, and histological technique in general is left to the special manuals. Each chapter concludes with a list of references.

The subject is approached through consideration of the cell wall, meristems, cell types, etc., with appreciation of the functional point of view rather than strict classification of tissues by their method of development. There is repeated recognition of progressive dedifferentiation and its various applications. The omission of all illustrations may lead to some difference of opinion. Obviously this permits the inclusion of more material per given number of pages and favors compactness. The plan should promote independent or inductive study rather than identification of material in the laboratory. Since the author stresses fundamentals and gives less attention to variations in given cells and tissues, the program will probably facilitate achievement under the guidance of a competent instructor.—ROBERT B. WYLIE.

Aquatic Phycomycetes (Exclusive of the Saprolegniaceae and Pythium). By FREDERICK K. SPARROW, JR. Ann Arbor: University of Michigan Press, 1943. Pp. xix+785. Figs. 637. $5.00.

For some time there has been increasing need for a book in English comparable with MINDEN'S *Kryptogamenflora der Mark Brandenburg*. This need has now been met by SPARROW.

The introduction covers such matters as phylogeny, occurrence, etc.; and precise information on how to collect, purify, and culture the saprophytic forms is assembled for the first time. In discussing phylogeny, SPARROW agrees with SCHERFFEL in believing that the Phycomycetes have arisen from the monads and developed independently along uniflagellate and biflagellate lines. The introduction ends with a key to the orders, of which there are eight of good standing, with a ninth of doubtful position. These orders are dealt with fully, except for the omission of the genera *Synchytrium*, *Physoderma*, and *Urophlyctis* (all parasites on land plants) from the Chytridiales, the family Saprolegniaceae from the Saprolegniales, and the non-aquatic members and *Pythium* from the Peronosporales. Apparently diagnoses of *Olpidium brassicae* and related species are omitted because of their parasitism on land plants.

The treatment of each order consists of a well-documented, terse introductory part in which the morphological and physiological researches are reviewed. This is followed by the systematic part, with keys and technical diagnoses, totaling 8 orders, 21 families, 108 genera, 475 species, and 10 varieties. Twelve of these genera and some 40 of the species were originally described by SPARROW. Among these diagnoses are many which were first published in various foreign languages. These have been translated, either literally or in modified form, and brought together in a way to make them usable by all English-speaking students.

Over half the book (369 pages) is devoted to the Chytridiales, after removing the Plasmodiophorales and Hyphochytriaceae from this order. This allocation of space is justified, for while all the other orders treated have been monographed previously in English, this is the first comprehensive treatment of the Chytridiales to appear in English and is far and away the most complete in any language. A list of substrata, a bibliography of about 720 titles, and an index complete the book.

The 637 figures, many of which are after SPARROW, are well chosen and beautifully reproduced. Binding and typography are excellent. The frontispiece shows photographs of DANGEARD, ZOPF, PETERSEN, and CORNU. This volume will doubtless remain the outstanding work in this field for a long time; even when superseded, it will still rank as a classic.—J. N. COUCH.

Timbers of the New World. By SAMUEL J. RECORD and ROBERT W. HESS. New Haven, Connecticut: Yale University Press, 1943. Pp. xv+640. Pls. 58. Maps 8. $10.00.

This volume is the culmination of more than 25 years of intensive investigation and is based on the Yale collection of woods, including about 20,000 Latin American specimens. Encyclopedic in nature, this work will be useful primarily to wood technologists, tropical botanists, and foresters, and of interest to explorers, importers, botanists, and laymen in general. It supplies much essential and interesting information in a logical and simple manner—much of it never before assembled in such an accessible form.

Thirty pages and several plates are devoted to gymnospermous trees, from *Araucaria* to *Taxodium*. Illustrations range from the Alerce (*Fitzroya*) of Patagonia to white pine in Utah and Douglas fir in Washington.

Angiospermous families, numbering 148, are also arranged alphabetically. Each family is briefly characterized, with emphasis on general distribution and gross features of stem, leaf, flower, and fruit. The American genera are listed and their economic importance noted. Specific qualities of the wood, such as color, specific gravity, grain characteristics, and cellular details, are summarized. The number of genera and species is indicated for each family.

More than 1000 genera are treated individually in most cases, with separate paragraphs for prominent species. When known, all essential qualities of the wood are given for each species, including density, compressibility, bending tolerances, elasticity, fiber stress, texture, durability, etc.

Frequent citations to recent botanical and technological articles are included. Naturally the discussion of such well-known genera as *Quercus* and *Acer* is limited to the more important commercial species, while the discussion of other genera summarizes all extant information. A comprehensive list of common names for each species for each nation or region is given.

Seventy-five photomicrographs of representative woods, a bibliography for each major geographic subdivision of the western hemisphere, lists of families classified according to special properties, and uses of bark, leaf, or timber, together with a complete index, enhance the value of this book.—PAUL D. VOTH.

Rio Mayo Plants: A Study of the Flora and Vegetation of the Valley of the Rio Mayo, Sonora. By HOWARD SCOTT GENTRY. Carnegie Institution of Washington Publication 527, 1942. Pp. vii+328. Pls. 29. Figs. 6. Maps 2.

Improved transportation facilities to some of the many parts of northwestern Mexico still unexplored by botanists, and the thorough work of the author, extending over 7 years, have resulted in this valuable floristic study. Rio Mayo originates in the high mountains of Chihuahua and flows southwestward through the State of Sonora, reaching the Gulf of Mexico during rainy seasons. Geography, climate, information concerning travel facilities, an ecological discussion of the thorn, short-tree, oak, and pine forests, a record of secondary succession on abandoned fields, and lists of cultivated plants with Mexican and Indian vernacular names precede the floristic list which constitutes the greater part of this volume. Collections number 3200, representing 1276 species and varieties of which 90 have been described as new. The interpenetration of subtropical and desert plants in this region is emphasized by this study. Photographs and typography are excellent.—PAUL D. VOTH.

Common Edible Mushrooms. By CLYDE M. CHRISTENSEN. Minneapolis: University of Minnesota Press, 1943. Figs. 62. $2.50.

Those who are interested in mushrooms from a culinary standpoint will find this volume helpful in determining species which may be eaten with safety. Tests for identification are reasonably direct and simple. The abundant clear-cut illustrations are purposeful.—E. J. KRAUS.

GENERAL INDEX

A

Acrophorus, development of sorus and prothallium 437
Algae, role of pyrenoids 633
Allison, F. E., Biochemical nitrogen fixation studies 63
Anatomical studies of roots of pine 443
Anatomy, and histology of Eureka lemon 288; of date fruit 426
Apple fruit, morphology and histology 3
Apples, respiration in storage 178
Arabogalactan, hydrolysis by Aspergillus 329
Arnon, D. I., Composition of the tomato plant influenced by nutrient supply 576
Aspergillus, hydrolysis of arabogalactan by 329
Atwood, Alice C., "Geographical guide to floras of the world" 189
Aurantioideae, chromosome numbers 602
Auxins, comparative activity of synthetic 281
Avery, G. S., Comparative activity of synthetic auxins and derivatives 281
Avocado blossom buds, differentiation 323

B

Baldwin, H. I., "Forest tree seed" 367
Bates, J. C., Varietal differences in anatomy of wheat grain 490
Bausor, S. C., Effects of growth substances on reserve starch 115
Beal, J. M., Cytological studies in relation to the classification of the genus Calochortus. III 553
Bean, affected by platinum chloride 161; mineral nutrition and light 133
Beeson, K. C., Effects of micro-nutrient deficiencies on growth and vitamin content of the tomato 495
Berger, J., Comparative activity of synthetic auxins and derivatives 281
Blair, Winifred M., Device for measuring entry of water into roots 152
Blake, S. F., "Geographical guide to floras of the world" 189
Bonner, James, Effects of application of thiamin to Cosmos 475
Borthwick, H. A., Influence of temperature on photoperiodic reactions in leaf blades of Biloxi soybean 612
Bose, S. R., Role of pyrenoids in algae 633
Buchholz, J. T., Obituary sketch of C. J. Chamberlain 369

C

Calochortus, classification of the genus in relation to cytology 553
Cave. M. S., Development of macrogametophyte of Miersia 185
Chamberlain, C. J., Obituary sketch 1, 369
Chandler, W. H., "Deciduous orchards" 188
Chemical changes accompanying development of peach 348
Chin, T. C., Cytology of the autotetraploid rye 627
Chlorophyll-protein complex 449
Chlorophylls *A* and *B*, effects of solvent upon absorption spectra 515
Chloroplast substance of spinach leaves 122
Chromosomal association, during meiosis in Dactylis 171; in clones of Dactylis 541
Chromosome numbers in Citrus 602
Citrus, chromosome numbers 602
Clark, H. E., Effects of growth-regulating substances on pineapple 639
Colchicine, effect on Colchicum and Chlamydomonas 50
Comar, C. L., Chloroplast substance of spinach leaves 122
Convolvulus, development of root system 417
Cooper, G. O., Microsporogenesis and development of seed in Lobelia cardinalis 72
Cornman, Ivor, Susceptibility of Colchicum and Chlamydomonas to colchicine 50
Cosmos, effects of thiamin 475
Couch, J. N., book review 651
Cranberry Glades, vegetation and substrate 371
Cryptomitrium tenerum 264
Cucurbita, anatomy 394
Cytological studies of Calochortus 553

D

Dactylis, chromosomal association during meiosis 171; variance and covariance of chromosomal association 541
Darlington, H. C., Vegetation and substrate of Cranberry Glades 371
Date fruit, anatomy 426
Defoliation of tomato plant 486
Differentiation of avocado blossom buds 323
Dissociations of cellular proteins and incipient drought 480
Dorf, Erling, "Upper Cretaceous floras of Rocky Mountain region" 494
Dormancy of spores of Phycomyces affected by growth substances 224

E

Eaton, S. V., Influence of sulphur on metabolism of black mustard 306; Volatile sulphur content of black mustard plants 82

Echinocystis, anatomy 394
Effects of, growth-regulating substances on shoot development of roses during common storage 26; growth substances on reserve starch 115; photoperiod on microsporogenesis in soybean 99; light quality and mineral nutrition on bean 133; micronutrient deficiencies on vitamin content of tomato 495; nutrient-solution concentration on growth of Marchantia 591; platinum chloride on bean and tomato 161; solvent upon absorption spectra of chlorophyll 515; sulphur deficiency on metabolism of mustard 306; thiamin on Cosmos 475; vaporous naphthoxyacetic acid on development of tomato 362; vitamins on germination of orchids 455
Ellis, G. H., Effects of micro-nutrient deficiencies on growth and vitamin content of the tomato 495

F

Fishman, M. M., Chlorophyll-protein complex 449
Floral stimulus in Xanthium, translocation 409
Ford, E. S., Anatomy and histology of the Eureka lemon 288
Foster, A. S., "Practical plant anatomy" 651
Frazier, J. C., Nature and rate of development of root systems of Convolvulus 417
Fruiting of tomato plant influenced by nutrient supply 576

G

Gaseous emanations from tomato fruit and defoliation 486
Gentry, H. S., "Rio Mayo plants" 652
Growth substances, effects of 26, 50, 115, 161, 224, 243, 281, 338, 362, 455, 475, 486, 639, 650

H

Hamner, C. L., Effects of platinum chloride on bean and tomato 161; Treatment of seeds with synthetic growth-regulating substances 338
Harris, D. G., Effects of solvent upon absorption spectra of chlorophyll 515
Haupt, A. W., Studies in Californian Hepaticae 264
Hayward, H. E., Device for measuring entry of water into roots 152
Hepaticae, Californian 264
Hill, Helen D., Variations in chromosomal association and behavior during meiosis in Dactylis 171
Histological responses of Vicia faba to indoleacetic acid 243
Hoagland, D. R., Composition of the tomato plant influenced by nutrient supply 576
Hoover, S. R., Biochemical nitrogen fixation studies 63

I

Iris fulva, cell size in developing ovaries 90

K

Karling, J. S., "Plasmodiophorales" 366
Katz, Elaine J., Pollen-tube development in Taraxacum 650
Kavanagh, Virgene and Frederick, Growth substances and dormancy of spores of Phycomyces 224
Kerns, K. R., Effects of growth-regulating substances on pineapple 639
Kraus, E. J., book reviews 188, 366, 367
Krug, C. A., Chromosome numbers in the subfamily Aurantioideae 602

L

Land, W. J. G., Obituary sketch 1
Lee, F. A., Chemical changes accompanying development of peach 348
Legume nodules, excised 63
Legumes, species relationships shown by electric mobility curves 449
Lemon, anatomy and histology 288
Lilium, occurrence of aneuploids 620
Lloyd, F. E., "The carnivorous plants" 494
Lobelia, microsporogenesis in 72
Long, E. M., Developmental anatomy of the fruit of the date 426
Lowenhaupt, Benjamin, Nutritional effects of boron on growth of sunflower 316
Lutman, B. F., book review 366
Lyon, C. B., Effects of micro-nutrient deficiencies on growth and vitamin content of the tomato 495

M

Marchantia, effects of nutrient concentration 591
Marth, P. C., Effects of growth-regulating substances on shoot development of roses during common storage 26
Measuring entry of water into roots 152
Meiosis in autotetraploid Secale 563
Mentha, spindle abnormalities 273
Meyer, S. L., Physiological studies on mosses. IV 128
Microsporogenesis and development of seed in Lobelia cardinalis 72
Miersia, development of macrogametophyte 185
Minor elements, review of Third Supplement 189
Minor, F. W., Biochemical nitrogen fixation studies 63
Mitchell, J. W., Effects of vaporous naphthoxyacetic acid on development of tomato 362
Morrow, Dorothy, Cell size in developing ovaries of Iris fulva 90
Mosses, physiological studies 128
Moyer, L. S., Chlorophyll-protein complex 449
Mustard plants, sulphur content 82, 306
Myers, W. M., Analysis of variance and covariance of chromosomal association in clones of Dactylis 541; Variations in chromosomal association and behavior during meiosis in Dactylis 171

N

Nelson, Ray, Spindle abnormalities in Mentha 273
Nielsen, C. S., Effects of photoperiod on microsporogenesis in Biloxi soybean 99
Nightingale, G. T., Potassium and phosphate nutrition in pineapple 191
Nitrogen fixation studies 63
Noggle, G. R., Effects of vitamins on germination of orchids 455
Northen, H. T., Relationship of dissociation of cellular proteins by drought to physiological processes 480
Nucleolar stain and nucleal reaction 639
Nutritional effects of boron on growth of sunflower 316
Nutrition, mineral, of bean 133; of pineapple 191

O

Olmsted, C. E., book reviews 188, 189
O'Mara, J. G., Meiosis in autotetraploid Secale 563
Orchids, germination and growth 455
Owens, H. S., Optimal conditions for the hydrolysis of arabogalactan by Aspergillus niger 329
Ownbey, Marion, Cytological studies in relation to the classification of the genus Calochortus. III 553

P

Palser, Barbara F., Histological responses of Vicia faba to indoleacetic acid 243
Parker, M. W., Influence of temperature on photoperiodic reactions in leaf blades of Biloxi soybean 612
Parthenocarp[illegible], affected by growth substances 639
Patterson, Sister Mary T., Cytology of apogamy in Polysticum 107
Peach, chemical ch[illegible]ges accompanying development 348
Peas, transplantation experiments 460
Petunia pollen, X-ray treatment 528
Photoperiod, effects on soybean 99, 612
Phycomyces, dormancy of spores affected by growth substances 224
Pine, anatomy of root 443
Pineapple, effects of growth substances 639; potassium and phosphate nutrition 191
Platinum chloride, effects on bean and tomato 161
Polysticum, cytology of apogamy 107
Preston, R. J., Anatomical studies of roots of pine 443

R

Raspberry canes, histological study 356
Ratajak, E. J., Optimal conditions for the hydrolysis of arabogalactan by Aspergillus niger 329
Record, S. J., "Timbers of the New World" 651
Reece, P. C., Differentiation of avocado blossom buds 323
Reed, H. S., "A short history of the plant sciences" 188
Regeneration in Physcomitrium 128
Respiration of apples in storage 178
Reynolds, Mary E., Development of the node in Ricinus 167
Ricinus, development of the node 167
Rick, C. M., Cyto-genetic consequences of X-ray treatment of pollen in Petunia 528
Riley, H. P., Cell size in developing ovaries of Iris fulva 90
Robbins, W. J., Growth substances and dormancy of spores of Phycomyces 224
Robbins, W. W., "Weed control" 367
Rohrbaugh, L. M., Effects of light quality on growth and mineral nutrition of bean 133
Root, measuring water uptake 152; system of Convolvulus 417
Rosenberg, H. R., "Chemistry and physiology of the vitamins" 188
Roses, effects of growth substances during storage 26
Rye, cytology of the autotetraploid 563, 627

S

Salisbury, E. J., "Reproductive capacity of plants" 366
Schopf, J. M., book review 494
Scott, Flora M., Anatomy of Echinocystis and Cucurbita 394
Secale, meiosis in autotetraploid 563, 627
Seeds of radish and oats treated with growth substances 338
Semmens, C. S., Nucleolar stain and nucleal reaction 639
Shalucha, B., Comparative activity of synthetic auxins and derivatives 281
Shull, C. A., book reviews 188, 189
Simonds, A. O., Histological study of raspberry canes 356
Skaling, P. E., Device for measuring entry of water into roots 152
Skok, John, Defoliation of tomato plant as response to gaseous emanations 486
Smock, R. M., Influence of controlled-atmosphere storage on respiration of apples 178
Soybean, effects of photoperiod 99; influence of temperature on photoperiodic reactions 612
Sparrow, F. K., "Aquatic Phycomycetes" 651
Spinach leaves, chloroplast substance 122
Spindle abnormalities in Mentha 273
Starch reserve, effects of growth substances 115
Stewart, R. N., Occurrence of aneuploids in Lilium 620
Stewart, W. S., Treatment of seeds with synthetic growth-regulating substances 338
Storage, apples and respiration 178; roses affected by growth substances 26
Sulphur content of mustard plants 82
Sunflower, nutritional effects of boron on growth 316

Susceptibility of Colchicum and Chlamydomonas to colchicine 50
Swanson, C. P., Spindle abnormalities in Mentha 273

T

Taraxacum, pollen-tube development 650
Tehon, L. R., "Fieldbook of native Illinois shrubs" 494
Temperature, influence on photoperiodic reactions of soybeans 612
Thomson, Betty F., Development of sorus and prothallium of Acrophorus 437
Tomato, affected by growth substance 362; affected by micro-nutrient deficiencies 495; affected by platinum chloride 161; composition influenced by nutrient supply 576; defoliation of plant affected by gaseous emanations 486
Translocation of floral stimulus in Xanthium 409
Transplantation experiments in peas 460
Tukey, H. B., Chemical changes accompanying development of peach 348; Gross morphology and histology of developing fruit of the apple 3

V

Van Dersal, W. R., "Ornamental American shrubs" 367
Varietal differences in anatomy of wheat grain 490
Vegetation and substrate of Cranberry Glades, West Virginia 371
Vicia faba, histological responses to indoleacetic acid 243
Vitamin content of tomato 362, 495
Vitamins, effect on growth of orchids 455
Voth, P. D., book reviews 494, 651, 652; Effects of nutrient-solution concentration on growth of Marchantia 591

W

Water entry into roots, device for measuring 152
Went, F W., Transplantation experiments in peas 460
Wheat grain, varietal differences in anatomy 490
Whitehead, Muriel R., Effects of vaporous naphthoxyacetic acid on development of tomato 362
Winter injury of raspberry canes 356
Withrow, Alice P. and R. B., Translocation of the floral stimulus in Xanthium 409
Wylie, R. B., book reviews 494, 651
Wynd, F. L., Effects of vitamins on germination of orchids 455

X

Xanthium, translocation of floral stimulus 409
X-ray treatment of pollen in Petunia 528

Y

Young, J. O., Gross morphology and histology of developing fruit of the apple 3

Z

Zscheile, F. P., Effects of solvent upon absorption spectra of chlorophyll 515